An Investigation of the Methods of Bacterial Technique, Preparation of Cultural Media, Cultural Characteristics, and the Classification of Bacteria.

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One engaged in the study of bacteria cannot but be impressed with the many difficulties attending the identification of the various bacterial species.

Not only is the literature upon the subject widely distributed among the different periodicals, memoirs and text books, but individual descriptions are, in the majority of cases, very incomplete.

I have called attention to the inadequacy of the various classifications and descriptions of bacteria in a former publication (1); inaccuracies and variations in method which have long been known to bacteriologists; the investigation of these subjects, however, is still very incomplete.

Up to the year 1901 practically no work had been done along the lines of systematic investigation of the variations occurring in cultural media, and of the necessity of taking steps to insure uniformity in method and technique.

In 1901 the results of an investigation undertaken by the laboratory section of the American Public Health Association were made public (2).

This work was conducted under the direction of Mr. Whipple (sometime Secretary of the Section) in the following manner: two cultures of bacteria representing species having quite different cultural and biochemical characters, were sent to a few of the leading bacteriologists associated with the American Public Health Association. Each investigator used his own methods of bacterial procedure and technique, and the results were forwarded to the Mt. Prospect Laboratory for tabulation.

The results were very discordant, and showed the greatest variations in the following respects:

A- the choice of characteristics considered necessary for a complete description of a species, that is, descriptions specific enough to separate the given organism from every other organism;

B- the great diferences as regards the same characteristic; in many cases the results obtained by different observers were diametrically opposed to one another.

This work led to another investigation, using as before organisms of widely different characteristics. The committee recommended a procedure to be followed in

detail by each observer to insure uniformity in the results.

The biochemical features, and in general the cultural characteristics were more constant than in the preceeding research. There were still, however, evidences that either there was a great personal factor in interpreting the results upon the different cultural media, or else the media were widely different in some respects.

To determine the latter point a final investigation was undertaken by Mr. Whipple, he himself making all the media and controlling the work in his own laboratory. He obtained gelatin from the lots used by the different observers, and in addition bought various samples in the open market. The material was made at once into nutrient gelatin according to the formulae prescribed by the American Public Health Association Report. Tests were conducted showing the more salient physical properties of nutrient gelatin: spissitude, melting point, reaction, chlorine content, viscosity, and specific gravity. To determine their relative values as nutrient media, each sample of gelatin was

seeded with an equal amount of a sample of Brooklyn tap water; the relative number of colonies developing, the proportion of liquefying to non-liquefying colonies and a comparison of the relative number of similar organisms developing upon each lot of media were carefully noted.

Finally, four species of bacteria, _Bacillus pyocyaneus_, _Bacillus violaceus_, _Bacillus fluorescens liquefaciens_ and _Bacillus_ '_C_' were inoculated into tubes of each medium, and incubated under the same conditions to determine the constancy of form of liquefaction, line growth, character of surface growth and presence or absence of a pellicle upon the liquefied portion of the gelatin.

The results of this elaborate series of studies revealed the same range of cultural characteristics noted in the work of the individual observers, and showed conclusively that cultural reactions and other characteristics upon nutrient gelatin were much less constant than was formerly supposed; that species whose descriptions find expression merely in some slight variation of growth upon gelatin can have no standing, unless very carefully controlled, and that the 'medium factor' is a problem of the first importance in future work in bacteriology.

The present investigation was undertaken to see if any further light could be shed upon these puzzling variations.

In the first place it is important to determine if there is agreement among bacteriologists as to the conception of what constitutes a given species: in other words in light of our present knowledge concerning the great variations which occur in cultural media, do the bacteriologists always isolate as *Bacillus coli*, for example, an organism which will always react in a certain definite manner upon our customary media? The most satisfactory method of elucidating this point is to obtain cultures of the 'colon bacillus' from different sources and work them out at the same time and under the same conditions upon the *same* lot of media.

If such uniformity of interpretation of the 'colon bacillus' exists, one will be in a better position to study the phenomena bearing upon the subject of variations occurring in cultural media under different conditions, and to explain their significance in connection with the subjects of classification and identification

of bacteria.

The research outlined below is an attempt to study some of these factors; their extent and relation to bacteriological diagnosis.

Outline of the Investigation.

Part 1. (pp. 9-83.)

Discussion of methods of bacteriological investigation including the methods employed in this research.

1. Preparation, composition, storage, and use of media; precautions and controls.

2. Preparation, preliminary cultivation and genealogy of cultures; seeding of media.

3. Methods of inoculation of media; controls and precautions.

4. Discussion of biochemical features.

5. Observations upon morphology, including a consideration of the 'hanging block' method of observing reproduction of bacteria.

6. Method of recording the characteristics, morphological, cultural and biochemical, by the decimal system.

Part II. (pp. 83-141.)

Investigation of the cultures of the same bacterial species from different sources upon the same lot of media under the same conditions; discussion of results, their application to classification of bacteria, etc.

Part III. (pp. 141-228.)

Investigation of the same species of bacterium upon different media under the same conditions in order to study

1. The influence of reaction of the medium.
2. The influence of concentration of the medium.
3. The influence of temperature and moisture.
4. The influence of the time-factor in the final results.
5. The influence of certain albuminous substances.
6. The constancy of interpretation of cultural phenomena by different observers of the same series of cultures, i.e. the 'personalfactor'.
7. The value of the various cultural and biochemical characteristics of bacteria for purposes of classification, based upon their relative constancy.

Part IV. (pp. 228 – 252.)

Study of the morphology of bacteria, especial emphasis being laid upon

1. The form and size.

2. Motility and flagellation.

3. Germination and form of spores in the cell.

4. Method of vegetative reproduction.

Part V. (p. 252.)

Summary, Bibliography, and Appendix relating chiefly to the composition of media used in the investigation, and a proposed form for standard tabulations of the characters of bacteria.

[Curriculum Vitae, p. 262.]

Part 1.

All the media used in this investigation were made according to two formulae; one recommended by the Committee of Standard Methods of Water Analysis of the American Public Health Association; the other used at the Johns Hopkins Medical School.

In order to have some standard for comparison of the different results, thirty (30) pounds of lean beef were made up into one large batch of agar, at the same time and under the same conditions as far as possible. Particular attention was paid to the time factor in cooking and sterilizing this material. Finally, the reaction of the whole mass was adjusted in one large vessel; of course the material was at the same time thoroughly mixed so that each and every portion had the same composition and reaction. The medium, which was prepared and sterilized under the same conditions, was stored in the dark in a large ice-box, where it was subjected to the same conditions of temperature, moisture and other physical conditions.

This medium, just mentioned, was designated as

Agar number 4. In every series of experiments, at least one complete set of inoculations was made upon Agar 4; direct comparison of different sets of results could be made through the mediation of this medium.

Successive series of results could thus be referred to this standard medium for comparison; changes in the cultural features of an organism when kept from generation to generation could be easily demonstrated.

Method of Preparation of Gelatin and Agar.

1. American Public Health Association Method. *

Step. Procedure.

1. Infuse lean meat 24 hours with the proper amount of distilled water (twice the weight of meat) in the refrigerator.

2. Weigh the infusion and add water to make up any loss due to evaporation.

3. Strain the infusion through cotton cloth.

4. Weigh the filtered infusion.

5. Add 1 % peptone (Witte's).

6. Warm on a water bath (?) stirring constantly,

until the peptone isdissolved; keep the temperature below 50° C.

7. Titrate and bring the reaction down to the required reaction with normal NaOH.

8. Heat on the calcium bath for half an hour,

9. Weigh and restore loss due to evaporation.

10. Titrate.

11. Adjust to the desired final reaction.

12. Boil five minutes over the free flame with constant stirring.

13. Restore loss due to evaporation.

14.*Filter through absorbent cotton, passing the filtrate through the filter until clear.

15. Titrate and record the final reaction.

16. Tube.

17. Sterilize.

* The above method is one introduced by Mr. Rickards, (A System of Stating Methods of Making Media), read at the Philadelphia Meeting of the Society of American Bacteriologists. The method was devised to simplify the methods of making the various cultural media; the foundation is broth: other media as a rule have this medium

as a basis. A few simple additions to the above formula in the appropriate places of the proceedure enable one to express very simply, concisely and definitely the desired steps.

† Made by using a saturated solution of calcium chloride instead of water in the double boiler. The advantage is that one obtains a uniform, higher temperature than is possible with water. The bath boils at about 106° C.

Nutrient Agar. Modification of the above formula.

Step 1. use equal weight of water instead of twice the weight as in the formula.

Step 2. -4 same.

Step 5. add 2 % peptone.

Step 7-8 add 2% agar jelly. (made by adding 20 grams of thread agar to water, dissolving with the aid of heat and adding at step 7-8 after cooling to 50°C.

Nutrient Gelatin.

Step 5. add also 10 % sheet gelatin.

It will be seen that any medium can be expressed in a few sentences in the above scheme; the change and place of change are very clearly indicated.

The Johns Hopkins Method.

Plain Nutrient Agar.

Formula;	Liebig's Extract of Beef.	2.5 grams.
	Peptone (Witte's)	10.0 grams.
	Sodium Chloride.	5.0 grams.
	Agar	15.0 grams.
	Water (tap).	1500.0 cc.

Proce~~e~~dure.

a- place the pan containing the water upon the furnace, shred the agar into this, drop in the meat extract, and stir until the agar is quite dissolved, removing the floating scum when necessary by skimming.

b- remove the pan from the fire, cool slightly and gradually 'dust' in the peptone and salt, stirring the while to facilitate solution. Replace the pan upon the fire and bring to a boil, and keep boiling and stirring until the lumps of peptone are dissolved.

c- adjust the reaction of the medium to alkalinity. This is best and most simply accomplished for ordinary work by the use of a 5 % solution of NaOH, employing as an indicator phenol-phthalein paper. When this test

paper strikes a pale pink color the reaction is to be considered correct.

d- remove the pan from the fire and cool the contents to 60° C. having in the mean time mixed two eggs in 150 cc. of water; stir in the eggs, replace the pan on the furnace, being careful to have a slow fire burning in order that the egg albumen be slowly but thoroughly coagulated. Refrain from stirring during this process. When coagulation is complete, weigh the pan and contents allowing 50 grams for each egg, and adjust the weight so that the contents will weigh exactly 1000 grams.

e- filtering; when the medium is ready, set up a glass funnel and wire paper holder in a filter stand. Fold a filter paper and moisten. Pour the contents of the pan through the paper, slowly.

f- sterilize 7 minutes at 15 pounds pressure in the autoclave.

Nutrient Gelatin.

The same as plain agar, substituting 120 grams gelatin for the agar.

Proceedure; have the water boiling; take a sheaf of gel-

atin leaves by one end and dissolve them by waving them to and fro in the boiling water. Cause the water to cease boiling and proceed precisely as in making agar, except that further boiling should be avoided. Filter, tube and sterilize, using 5 minutes exposure instead of 7 as in the case of agar.

Storage of Media.

Mention has already been made of the general method of storage of media; all media were placed in the ice-box as soon as possible after preparation. The ice-box was large enough to accommodate 100 litres; the advantage of using such commodious quarters is three-fold. The conditions of light, temperature and moisture (gaseous environment) are practically constant.

The necesity of placing gelatin in a cool place immediately after sterilization has been shown by Whipple (loc. cit). The melting point is higher than if the medium were allowed to cool slowly at room temperature. In fact it is advisible to place the tubes containing gelatin in cool running water as soon as possible after autoclaving, a proceedure invariable carried out in this work. Differences of as much as three

degrees in the melting point are often noted when this cooling process is practiced.

The effect of long continued storage might theoretically produce changes of reaction, consistency, concentration and food value; the possibility of such changes were kept constantly in mind during this investigation, but if such changes did occur, they were too slight value to be detected by ordinary methods of research.

Agar number 4, referred to several times already, did not change in reaction after four months, nor were there any evidences of evaporation.

Determination of the Chemical Reaction of Media.

The reaction of each and every lot of media used in this work had its reaction carefully determined. The method presented below was the one adopted, and gave excellent results.

Analyses were made in triplicate. If the medium was solid, three tubes were melted upon the water bath; in no instance was the older and more convenient method of heating the tube in the free flame until the medium became fluid employed. The atmosphere above the water

bath is practically saturated with moisture,so there was a minimal loss be evaporation while the tubes were being melted.

After the sample was thoroughly fluid, and had been carefully shaken to distribute equally the moisture, 5 cc. were removed with a clean 5 cc. pipette and placed in 45 cc. of boiling water,(distilled water),contained in a clean porcelain evaporating dish. (The water was tested to see if there was any acid or alkalai present as a measure of precaution).

The mixture was boiled for one minute to expel carbon dioxide and 1/2 cc. of a 0.5 % solution of phenolphthalein solution was added. The flame was removed,and 10 cc. of N/20 HCl were added; then the solution was boiled again for one minute, and titrated boiling hot with N/20 NaOH solution. to the appearance of a faint but distinct pink color.

The The HCl was added because the reaction of many of my media ran between + 0.2 to - 0.2 cc. of normal alkalai to the litre. this would in the one case require a fraction of a cubic centimetre of alkalai

to make the solution neutral, and in the other event the reaction was already alkaline.

Furthermore, the error in adding a fraction of a cubic centimetre is much greater in porportion than if a larger quantity is required. Summarizing these factors the procedure outlined is the most logical and the most accurate and was used in all determinations involving the reaction of media.

N/20 solutions were used in preference to N/10 solutions because a larger quantity in porportion to the concentration is needed to bring about a reaction, than if stronger solutions were used. The practice of using 10/N solutions furthermore, requires 10 cc. instead of 5 cc. to be withdrawn every time one wishes to make a titration. 10 cc. is too large a quantity when one makes three determinations upon a litre of medium.

If the medium is highly colored, the color will be less in a 5 % solution than in a 10 % solution. This is another argument for the use of weaker mixtures.

There was some difficulty in making duplicate analyses, because the end point is not absolutely sharp. If one makes a control, using the same amount of material,

but having the reaction definitely acid, one can have a definite end point for comparison. The dishes in which the control and actual determinations should be matched in color. When these conditions are fulfilled, one may make a series of titrations and have them all check within two tenths of one percent, the usual error allowed in ordinary volumetric work.

Before the final burette readings are made, it is advisable to allow the burettes to drain for two minutes. This is particularly important after a burette has been used with caustic solutions for a time; the tubes have a tendency to 'lag' in draining.

All standard solutions were stored in glass stoppered bottles; the caustic N/10 solution, used in titrating daily, was protected from atmospheric agencies by causing all the air that entered the bottle to pass through (1) a 20 % KOH solution, then through a drying tube filled with freshly fused lime. The air was furthermore passed back fom the burette to the reservoir, so that a minimal amount of fresh air was introduced. The precautions taken were amply sufficient to protect the

standard solution.

I was able to check up the standard solutions from time to time with solutions of known strength, furnished me through the kindness of Dr. Abel of the Department of Physiological Chemistry.

It is possible, and even probable that the precautions outlined above were totally out of porportion to the final accuracy of the work; physicists state that if one component of a series of measurements is accurate to (say) 0.5 %, it is not only unnecessary, but actually inaccurate to make other determinations in units of greater precision than 0.5 %. There are no data available upon the work carried out in this research, and the writer has erred on the side of too great accuracy rather than run the risk of error in the opposite direction.

Final Preparation of Media for Use.

Besides the necessity of constancy in the chemical and physical properties of media, two other factors are desirable in order to obtain uniform results; namely, sterility and freshly prepared surfaces upon which organisms

are to be grown.

Agar slants should always be freshly slanted prior to use in order that the moisture shall be uniformly distributed, and above all there shall be enough moisture upon the surface to furnish a suitable environment for the culture. Too much moisture, however, is undesirable; immediately after the agar is slanted, there is a contraction of the medium resulting in the mechanical expulsion of broth; time must be allowed for this to drain to the bottom of the tube; otherwise the growth of the organisms will be a spreading one, and be non-characteristic. If agar slants be placed in the thermostat for 12-18 hours immediately after slanting, then inoculated, one can remove any doubts as to sterility, and at the same time have a surface free from excessive moisture.

If the medium has become contaminated during the process of slanting, which may happen if the water bath is boiling vigorously and water is spattered upon the cotton plugs, the organisms will develop in the thermostat; when the tubes are given their final inspection

before inoculation, the growth will usually appear either upon the slanted surface or in the water of condensation. In the latter event, the fluid is turbid instead of being clear as is the case in good media.

An unforseen advantage resulted from the preliminary, incidental incubation at 37°; all the organisms investigated in this research grew well at body temperature, and repeatedly it was found by actual experiment that the cultures developed more rapidly during the first twenty four hours upon media previously kept in the thermostat than did parallel cultures upon media that were kept in the ice box.

Gelatin Stab Cultures.

If gelatin be allowed to remain in places where evaporation can take place, the surface becomes sunken-concave- and the moisture disappears to a considerable extent just beneath the surface. Incidentally, a surface tension results, which is most unpleasant when one wishes to inoculate such a tube with a platimum needle. The needle disturbs the equilibrium, the tension then man-

ifests itself by the production of unsightly fissures in the medium. The fissure may form at once, or after the lapse of a few minutes. Formations simulating gas bubbles frequently result, which may lead to error; besides, the characteristic line and surface growth may be destroyed by these formations.

The simple expedient of remelting and resolidifying the gelatin will suffice in every case to obviate this defect. Media stored in a cool, moist atmosphere will not, however, need such treatment.

Technique of Inoculation of Cultures; Preliminary Cultivation; Genealogy of Cultures.

When this work was undertaken the necessity for some simple, efficient means of keeping the history of the cultures was recognized. The most logical method, probably, is that of Pickards (3) . By a very simple system one may find out with a minimal expenditure of time the dates of inoculation, the media upon which transfers have been made, and a complete history of the organism from the start.

An example of the use of this system will make the procedure clear, and illustrate the advantage of such information, especially in connection with the subject of preliminary cultivation.

For convenience, a slight modification was adopted. The different species of bacteria used in this connection were designated by letters of the alphabet, e.g. A represents _Bacillus typhosus_ , B represents _Bacillus coli_, and so on. To indicate different strains of _Bacillus typhosus_ subscripts were appended to the appropriate letter, so that three different races of the typhoid bacillus would appear as A_1, A_2, A_3.

A decimal point is placed after the _specific_ portion of the complex; the figures to the _right_ of the decimal point are concerned wholly with the history of the culture: as has already been explained, the figures to the _left_ of the decimal point indicate the source of the particular culture, the species and matters relating to its isolation, etc.

Each time a transfer is made from one of these cultures, a numeral is added to number or numbers already

present at the right of the decimal point. If only one transfer is made, a "1" is added in the first place of decimals to the right of the last one used. If two transfers are made at the same time, the first receives the "1" the second is indicated by a "2". If three are made at the same time, the numbers would read 1,2,3, etc. Example: A_1. is a typhoid culture obtained from Laboratory X. The past history may or may not be known. When this culture is received, it is immediately transferred to an agar slant, and to a broth.

A_1 is the original culture. date X11/5/'02.

A_1.1 is the broth transfer. " X11/6/'02.

A_1.2 is the agar transfer. " X11/6/'02. (made at the same time. As the figures increase in complexity at the right of the decimal point, they may be abbreviated by the use of coefficients; the coeffient will indicate the number of successive <u>like</u> numbers in sequence. Example. A_1.1112 would be written $A_1.1^4 2$; A_1.11123111 would appear $A_1.1^3 231^3$ and so on.

Cards have been printed in which such data may

be recorded; the complete history of a laboratory culture may be found by going back from one number to another until the original card and originalhistory are found. The method is of the greatest value to bacteriologists engaged in systematic study of species, and the writer has found it invaluable furnishing as it does a method applicable to any and all organisms, whereby one may record in an available form all data relating to their history, etc.

Preparation of Cultures for Inoculation upon Cultural Media.

Several points must be provided for before one can hope to introduce bacteria into nutrient media and obtain the best and most constant results; the culture must be pure, be in an active state of vegetative reproduction, and should have been on artificial media long enough to insure constancy of reaction. (The question of freshly isolated cultures from water supplies, and their growth as compared with the same cultures after they have been grown upon artificial media need not apply here. The latter case has its value no doubt in determining

roughly the viability of the organism, but is hardly applicable in strictly first class species work.

When a culture was received for investigation, it was transferred at once to slant agar, and after growth had appeared upon the agar, plates were made. From the plates new slants were obtained, and a fresh series of plates poured. Even this elaborate proceedure in one instance failed to give a pure culture; a culture of Bacillus pyocyaneus persisted in giving a few gas bubbles, and was finally, after three trials, shown to be associated with a Bacillus coli. The culture was from an autopsy, and was transferred every day, including the plating for over a week. If the culture had been allowed to remain upon one medium, preferably broth, the pyocyanin might have dissolved the colon bacillus, and left a pure culture. A similar experience was found during the work upon the Shiga bacillus, during the work in the summer of 1903; upon two occasions, Bacillus dysenteriae showed gas formation after a lapse of two weeks, whereas the original culture had given perfectly typical react-

ions. This was due to the fact that a very small colon colony happened to lie beneath the Shiga colony, and a portion of the colon organisms were removed with the Shiga bacilli. The Bacillus coli was not present in large enough numbers to overcome the Bacillus dysenteriae at first, but being a more hardy organism, gradually outgrew the latter bacteria. From each of these mixed cultures the writer was able to isolate both species of bacteria by the plate method without difficulty. This is merely another example of the great difficulty one has in separating the various members of the gastro-intestinal bacteria from one another.

Preliminary Cultivation.

Fuller and Johnson (4),(5) , introduced this very important proceedure into bacteriological investigations. In fact we owe to these two men the introduction of what may be called "business methods" in bacteriology. They were among the first to investigate systematically the cultural media to see the relative value of such material from the stand point of constancy of growth.

Their original method of preliminary cultivation

included the growth of an organism upon an agar slant, a transfer to broth, gelatin plates and finally back to the agar slant, from which the customary inoculations were made. The organisms were allowed to grow upon each of the media for three days before they were introduced into any new media. By this method, they succeeded in evolving cultures which would react much more quickly and more certainly upon all the media.

The work was done upon water bacteria, hence a slight modification should be made for pathogenic bacteria, and those organisms growing best at the body temperature. Weston and Kendall (8) introduced the successive transfers into three or more successive broths, a method which increased the constancy of reaction, even with the water bacteria. Cultures treated to the three broth method grew with the greatest rapidity, and in a few instances, motility was greatly exalted.

Duval and Schorer claim to have exalted the motility of Bacillus dysenteriae until it became more or less actively motile, and they finally succeeded in staining flagella upon the same. The writer has conducted parallel

cultures, giving to one strain the preliminary cultivation, while the other 'daughter' culture was simply transferred from the original culture once. In each instance, except with certain anaerobes, there was marked difference in the rapidity with which growths appeared upon various media, and in general there was also a decided tendency for the cultures having preliminary cultivation to form growths which were very much more constant in their appearances.

It should be remembered that preliminary cultivation is not a hard and fast procedure; we are dealing with living things, which will react favorably or otherwise to changes and differences in our media which are too minute to be recognized by our present theoretical methods. What will suffice for one organism will be quite unsuccessful with another species. A general rule may be made, however; grow the organisms several successive times upon the optimum fluid medium. At least three transfers should be made. The growth should take place at the optimum temperature for the particular species of organism under consideration.

Preliminary cultivation assures one, then, that the cultures are pure (plated out twice), and that they are in excellent condition to give their characteristic reactions and growths upon the customary media. While preliminary cultivation cannot furnish conditions as they are in nature, it is the best that can be done with our unnatural -artificial- media.

Seeding Cultural Media.

Media are seeded preferably from fluid media rather than solid media because other things being equal, the growth of bacteria is more luxuriant in fluid media, and the bacteria are more or less equally distributed in a fluid medium. Hence, if one removes an oese of culture from (say) a broth tube, after shaking the same carefully, one will be able to duplicate the quantity removed by taking out another oese of material. On the other hand, if one removes an oese from a slanted surface, one will not be able to remove another oese or at least, several oeses each containing relatively the same number of organisms.

If approximately equal amounts of culture are added to each medium, the resulting growths will at least

have the advantage of being readily seeded at the start. If one wishes to compare growths at different times, the more factors one can have that are reasonably constant, the more nearly comparable the results. The further advantage in using a fluid culture as the source of the inoculating material is that one can make plates more readily with a liquid mixture than if one had to emulsify the solid growth and use the emulsion.

One should use a platinum needle instead of a loop, except in rare instances; if one's cultures are in good condition, enough material will be removed with the needle, and when one comes to sterilizing the platinum, the unsightly spattering which is of necessity an accompaniment in hasty sterilization, is avoided by its use. Slant cultures are more constant in their characters, and much more characteristic and less spreading if the needle is used in preference to the loop. Finally, great care must be taken to sterilize the desk after one has sterilized a loop in the flame. The spattered material may contain bacteria which are not killed, and which if left upon the desk would dry, and become disseminated through the air. It is quite possible that some of the enexplain-

ed laboratory infections may be due to negligence of this kind.

Certain other precautions were also taken. The cotton plugs of tubes were always burned off to insure the removal of dust, spores of bacteria and of moulds. The lips of tubes were flamed before a needle was introduced into them, or before any culture was removed from them. Finally, the needle was never introduced into the stock culture after making an inoculation into another tube, without its being resterilized. One may by some accident or oversight have a contaminated tube into which the inoculation was made; the contaminating organisms would be transferred back into the stock culture unless the above precaution were taken. Attention to these details together with the policy of incubating all media (except gelatin) in the thermostat will in the great majority of cases prevent contaminations, and give results which will be as uniform in character as the present methods will permit. Scrupulous attention to minor details, while involving a considerable expenditure of time, is more essential in bacteriology than in almost any other field, if one expects to get trustworthy results.

Biochemical Characteristics.

Fermentation, Indol, Nitrite, Free Ammonia Production.

Theobald Smith was the pioneer in exact fermentation work as applied to bacteriological research. He pointed out (7), (8), that four important facts were to be learned by a judicious use of the fermentation tube.

1. gas production.

2- gas ratio; relation of carbon dioxide to other gases.

3- acid production; acid fermentation of carbohydrate without the production of gas.

4- anaerobiosis; the ability of an organism to grow in an atmosphere practically devoid of atmospheric (free) oxygen.

Dr. Smith emphasizes the factors above enumerated as being of constant occurrence in certain bacteria, hence of diagnostic importance in bacteriological diagnosis and classification.

In his admirable paper attention is directed to the form and dimensions of the fermentation tube; these particulars have much to do with the success or failure of the results obtained by their use. The bulb should be of sufficient size to hold all the liquid in the closed arm in addition to that already present in

the bulb; there should be a constriction between the closed arm and the bulb(the neck) and the angle sustended between the closed arm and the bulb should not be too great (too acute) or difficulty will be experienced in filling and cleaning the tubes.

In addition, it should be noted that the constriction at the 'neck' already alluded to should not be too small, or one cannot fill the tubes: onthe other hand it should not be too large, in which event the anaerobic conditions become unreliable.

It is claimed that methylene blue and litmus will remain permanently decolorized, as long as there are no air bubbles in the closed arm. As soon,however, as air bubbles get in, there is an immediate oxidization of the dye with a return of color. (change of the leuco-dye by oxidization to the colored product).

If extreme precautions are used, particularly by heating the tubes in flowing steam just before using, one may be sure that anaerobic conditions will obtain, provided that the tubes are of the proper dimensions and form.

Anaerobic conditions in the closed arm.

With the fermentation tubes in ordinary use among bacteriologists, that is to say, the tubes offered for sale by the majority of manufacturers, there is usually, immediately after sterilization, no dissolved oxygen in the closed arm. Atmospheric air (and nitrogen) will, however, tend to diffuse in; there is a free surface of liquid in the bulb, and the liquid is unsaturated with the atmospheric gases. Air will be dissolved until the partial pressures of the oxygen and nitrogen are the same in and out of solution; the amount of each dissolved will depend upon the partial pressure, temperature, etc. As soon as oxygen is dissolved in the bulb, there will be a condition of affairs such that one portion of a fluid has dissolved in it a soluble substance, while the other portion (closed arm) is deviod of the dissolved substance. The equilibrium will be finally established by the diffusion of oxygen (and other atmospheric gases) into allportions of the fermentation tube. This will mean that there will be oxygen in the closed arm.

The amount of dissolved oxygen; the coefficient of absor-

ption in water (or broth) is 0.026.

There is in atmospheric air about 20 % oxygen. Hence in 100 cc. of broth there will be (leaving out temperature, barometric pressure,etc) about 0.026 x 20 % x 100 = 0.52 cc. of dissolved oxygen. The closed arm contains from 17 to 25 cc. of fluid, hence there will be an appreciable amount of oxygen in the closed arm; a large quantity when one considers the size and consequent individual need of a bacterium. There is, then after a longer or shorter time, a measureable amount of oxygen in the closed arm of the fermentation tube, where, theoretically, there should be anaerobic conditions, lack of oxygen.

In the bacteria, as in all living things, there is no hard and fast line between any two similar characteristics, but one gets all gradations between,although the majority may be the one or the other. The same condition holds with respect to growth in the closed arm of the fermentation tube; the majority grow well or not at all in this place, but there are forms which at times show a decided growth, at other times show no growth, and this group is variable one, both with respect to the differ-

ent species at the same time, and the same species at different times. It is precisely this rather large group of bacteria that cause confusion by their behaviour in the closed arm. Repeatedly I have noted this phenomenon, that a species would grow fairly well in one tube, and at another time, while the conditions are nearly the same, the organism will not grow at all. The explanation is probably the one elucidated above, the variations in the amount of oxygen at different times in the closed arm. Since the growth in the closed arm is taken to mean the capacity of an organism to grow anaerobically, one should make the test under the most favorable conditions, paying especial attention to the recent sterilization.

Gas Production and Gas Ratio.

Most bacteriologists are familiar with the variations in the amount of gas produced by a certain kind of bacterium at different times, even if the culture has had the same preliminary cultivation, and it is grown upon the same kind of medium. These same variations may also appear if one determines the gas ratio.

Some bacteriologists claim to obtain constant results

both with respect to gas ratio, and total gas produced. There are two explanations possible of the variation in gas ratio: either the organism forms carbon dioxide (soluble in caustic solutions) and gas insoluble in caustic solutions in different relative amounts at different times, or else, and this is more reasonable, the relative diffusibilities of the gases (principally hydrogen, carbon dioxide, and occasionally methane) determines the ratio. Hydrogen diffuses with much greater rapidity than other gases likely to be formed by bacterial activity; if the determination be made while bacterial activity is at its height, one would a priori expect different values for gas ratio than if the determination were made some time after the action ceased. We have not only the difference due to diffusion, but also the question of reaction between the carbon dioxide and any chemical products that might be formed, and finally, the question of relative solubilities. Such a large number of variables will amply explain the discordant results obtained from time to time, and justify one's questioning the value of gas ratio and the total amount of gas formed, as a criterion for the differentiation of bacteria.

Acid Production in Fermentation Solutions.

Many bacteria which do not ferment saccharine solutions with the production of gas will never the less act upon the carbohydrate producing acid. This feature has been made one of the criteria for division of bacteria into groups by many bacteriologists.

Meat 'juice' or meat extract are used in fermentation solutions, and together with peptone, furnish the nitrogen from which the bacteria may derive their nutriment. During the process of making media, most of the proteid of the meat 'juice' is coagulated, but there is a small amount left; this amount depends probably upon the reaction of the finished product, and exists as acid albuminate, because the saccharine solutions are always slightly acid. The meat extract on the other hand contains relatively small amounts of proteid, but much creatin, xanthine bases and a large amount of sodium chloride.

Before discussing the bearing of the composition of the fermentation solution upon its value as a medium for bacterial diagnosis, it will be well to define clearly the four great actions of bacteria upon organic material

suitable for their growth and nutriment.

These actions as ordinarily considered are;

1- hydrolytic splitting, with a consequent breaking of the molecule into simpler molecules, after adding on hydrogen and oxygen in the porportions to form water. (Sometimes there is apparently a reversal of this action, with a reforming of the original molecule, as occurs in the action of maltase; maltose is reformed from two molecules of dextrose).

2- oxidization of methyl groups to carboxyl groups.

3- splitting off of carboxyl groups as carbon dioxide.

4- splitting off amino nitrogen as free ammonia.

The final reaction which obtains in an organic solution in which bacteria have been growing will depend upon the relative amounts of acid or alkalai produced during the period of incubation of the culture. The changes in composition of proteid substances by bacterial action are relatively greater than in any other pabulum. The vast majority of bacteria will act upon proteid, and produce deep seated changes, and as a rule, the alkaline products predominate in the end products.

The end products referred to in the last paragraph cannot but be of considerable importance in connection with the action of bacteria upon carbohydrates. If the bacteria act upon the proteid and produce alkaline products, and at the same time act upon the carbohydrate and produce acids, the acid and alkaline compounds will tend to neutralize one another, and unless careful chemical analyses are made, it will be impossible to tell what portion of the end products are from the carbohydrate, and what came from the proteid. This is a serious objection to the use of reaction in saccharine solutions in bacterial classification. We have already seen that the predominating substances resulting from the bacterial decomposition may be alkaline, while the carbohydrate give rise to acid products in bacterial decomposition. There is another phenomenon which is frequently observed, namely that the closed arm of the fermentation may be acid in reaction, while the bulb may be acid for a day or two, then become alkaline. The longer such a tube is allowed to remain before testing the reaction, the more alkalai there is produced in the bulb and the less acid the reaction in the closed arm. Obviously the reaction which

one obtains will depend upon the time at which the determination is made, and the portion of medium from which the determination is reckoned. If the portion in the arm be chosen, the reaction will be acid, while it may be alkaline in the bulb. The greatest care, then, must be taken if one records the reaction of a saccharine solution to state whether the portion for analysis were taken from the closed arm, the bulb or a mixture of the two.

If one uses a fermentation solution made without either meat juice of meat extract, and adds peptone to furnish the nitrogen for bacterial growth, one will eliminate many of the acid and alkaline organic compounds; for some reason that is not perfectly clear, peptone does not ordinarily give rise to any considerable amounts of acid and alkaline products when acted upon by bacteria.

A fermentation medium, then, composed of sugar and peptone will amply suffice as a food for bacteria; the organic acid and alkaline compounds will be reduced to a minimum as far as the peptone is concerned, and the resulting reaction will more nearly depend upon the character of the action of the bacteria upon the carbohydrate.

If there is a definite acid reaction, one will be quite safe in assuming that the acid was formed as a result of the bacterial action upon the saccharine constituent of the medium. Finally, it should be stated that the majority of the bacteria will grow readily upon this medium. All bacteria that I have investigated, over a hundred species, grow quite as luxuriantly in this medium as they do in the fermentation medium as it is ordinarily compounded. There is in a good sample of peptone, practically no fermentable substance; one does not have to prepare "sugar-free" media, introducing variable amounts of the decomposition products of the colon bacillus if one employs the peptone sugar medium instead of the meat juice peptone carbohydrate medium.

Concerning the amount of carbohydrate added to a fermentation solution; it is a well known fact that carbohydrates produce great osmotic pressures in solution. The bacterial cell is a semipermeable membrane. We have ideal conditions, then, for the osmotic pressure to be manifested upon this membrane. Appert, the Frenchman who introduced the art of conserving various perishable foods,

employed rather concentrated saccharine solutions for this means: the bacteria preexisting in the material were not killed, at least for some time, but were prevented from growth by the abstraction of water from their interior. This was a true case of plasmolysis.

The same possibilities exist in the case of fermentation media except that the concentration of sugar is not more than 2 % whereas it was about 10 % in the case of the preserves. One percent of any sugar is ample for the determination of the various characteristics of bacterial action in such material, and it would be much better if bacteriologists would adopt the lower standard when making their media.

The ability of bacteria to withstand the plasmolytic action of sugars is very variable. For example I found an organism in sugar in Louisiana which would not only grow but grow luxuriantly in saccharine media where the sugar content was as high as 18 percent. In one instance the 'clarified juice', that is, the juice of the sugar cane which has been treated with lime to remove the nitrogenous material, settled, and the clear liquid drawn

off, was stored in a tank holding about 4500 gallons. The fires were allowed to go down, and the juice could not be treated at once; during forty eight hours the entire mass, which only two days before was as limpid as water, became a slimy, sour smelling viscous fluid, due to the change of the saccharose to a carbohydrate closely related to the starches and called dextrane. The concentration of sugar in the first instance was about 15 percent.

On the other hand, certain organisms cannot live in concentrations of as much as 2 percent.

To summarize what has been said about the fermentation tube; it must have a definite construction, particular attention being paid to the relations of the capacity of the bulb the constriction of the neck, and the angle subtended between the closed arm and the bulb. The gas ratio and the amount of gas produced are under ordinary conditions subject to considerable variations. The question of anaerobic conditions in the closed arm is subject to variations; these variations can be controllable if one takes the precaution to sterilize the tubes

immediately before use, to drive off all the dissolved oxygen. The reaction depends upon the time at which the observation is made, and upon the relative amounts of protein (albuminous) and carbohydrate substance there are in the solution. Finally, the relative luxuriance of growth between different species is, other things being equal, a direct expression of their relative ability to resist the plasmolytic action of the sugars, and their ability to derive their nutriment, particularly with respect to carbon, from the carbohydrate.

The writer has been in the habit of using the fermentation tube merely as a convenient means of demonstrating the gas production of bacteria. The question of anaeriobiosis is determined by growing the organisms in some inert gas, as hydrogen; if one adds litmus or methylene blue to such a culture medium, the dye will be decolorized if the conditions are strictly anaerobic. One has, then, an absolute check on anaeriobiosis, both with respect to the absence of oxygen and the growth or lack of ability to grow on the part of the organism. The reaction is best determined by direct titration of a

medium having the same composition as the medium in which bacteria are grown in the fermentation tube. In the reaction determination, however, the tube employed is an ordinary test tube, so that all differences in reaction between the bulb and closed arm will be eliminated.

The writer has also devised a fermentation medium based upon the 'semi-solid' medium of Hiss. This medium has the following composition;

peptone, 10 grams.

carbohydrate, 10 grams.

water, 1000 cubic centimetres.

agar, 5 grams.

litmus;added until a faint blue color obtains.

This medium is intended to show presence or absence of gas production, and the reaction of a bacterium upon any carbohydrate in which the organism may be grown. The medium is viscous enough to prevent the escape of gas bubbles, thin enough to have almost all the advantages of a liquid medium, and finally to allow one to determine at any time the reaction of the culture.The objection to this medium is that some bacteria will reduce the litmus.

This difficulty may be overcome by the addition of fresh litmus at the time the final determination of reaction is made. A large series of comparative tests have been made using this solution and the regulation fermentation solution in fermentation tubes, and with the exception of showing the anaerobic relations of the bacteria, (which are more or less inconstant) has checked up remarkably well with the fermentation tube. The reduction of the litmus is an important point; many bacteria reduce litmus constantly, while others have no apparent reducing power.

Indol Production and Detection.

Indol is one of the 'primary decomposition products' of proteid decomposition; it may be obtained by the action of certain chemicals, digestion or the action of certain species of bacteria acting upon proteid material.

Indol is comparatively easy to demonstrate in solutions; it is produced by many species of bacteria, but not all, and is consequently used as a confirmative test for certain bacteria.

Methods for Detecting Indol.

Cultural media of two sorts are used in the indol test; one a sugar free bouillon, which is simple bouillon

made in the usual way, and to which a culture of *Bacillus coli* has been added and incubated over night to act upon the carbohydrate that may be present; the bouillon is sterilized again, and in this condition is free from sugar. The other is the socalled Dunham's solution. It is composed of one percent peptone, to which has been added one half percent of salt. Many bacteriologists prefer to make the Dunham's solution without salt. The salt is added apparently, to facilitate the solution of the peptone, and with our modern peptones this step is quite unnecessary.

The medium must in any event be free from carbohydrate or other reducing substance, because such substances produce decomposition products which are inimical to indol formation. There is diversity of opinion as to the action of carbohydrates and similar substances upon the production of indol; some claim that the sugars give rise to acids, which prevent the formation of indol, others make the assertion that the indol production is essentially a process of reduction, and that the carbohydrates, being more easily reduced, prevent the characteristic action

taking place.

Chemical Properties of Indol.

Indol has the formula:  it will be seen that it is a condensation product of a benzene and a pyrrol ring. There is another possible formula, an isomer, of indol; in which the hydrogen of the imino group occupies a position adjacent to the nitrogen in the pyrrol ring, thus;  this compound is called isoindol. Its properties are not well known, and it seems probable that it has little or no importance in bacteriological work.

Indol gives certain characteristic reactions;

1- When heated slightly with picric acid, it forms long red needles.

2- Many indol derivatives give a cherry red color when their fumes are allowed to play upon a pine shaving moistened with concentrated hydrochloric acid. The compound liberated is pyrrol. An alcoholic solution of indol will give the same reaction.

3- Indol crystallizes from water; it is readily volatilized from an aqueous solution.

Finally, indol gives Legal's reaction; if a solution containing indol be treated with an alkaline solution of sodium nitroprusside, the solution becomes a deep reddish violet. On acidifying with hydrochloric acid or acetic acid, the color changes to a blue.

Indol is insoluble in water, soluble in anilin and alcohol. It unites readily with nitrous acid, forming nitroso-indol. This same reaction occurs if the sodium or potassium salt of nitrous acid are used. The routine test for indol is based upon the nitroso-indol reaction. This compound forms a red-brown color if treated with sulphuric acid. The test is carried out in the following manner; one adds to a Dunham's solution in which bacteria have been grown, a drop of acid (sulphuric) for every cubic centimetre of culture; if a red-brown color develops, indol is present, and in addition, the organism has reduced nitrate to nitrite in the solution. If no color develops, a cubic centimetre of 0.2 percent sodium nitrite is added. If now the characteristic color develops, indol (not nitroso-indol) is present. It is absolutely necessary to make careful control tests, incubating them with the culture tubes.

There are four ways in which the above mentioned reaction are carried out; the procedure varies in different laboratories.

1- the culture and the acid are heated to 100° Cent. upon the water bath.

2- the culture and acid are heated to 37° Cent. in the thermostat.

3- the culture and acid are kept at room temperature, 20° Cent.

4- the culture and acid are kept in the ice-box, and allowed to become 'stone cold' before testing.

The time allowed for a positive reaction also varies; of course heat facilitates the reaction, and cold to a certain extent retards the same, but all are practically agreed that two hours at the most will suffice at the lower temperature, and correspondingly less time should be allowed at higher temperatures.

Gruber and Francis (9) have introduced a modification of the routine method for testing for indol;they claim that their modification increases the sensitiveness and sharpness of the reaction. They proceed in the following manner:

the nitrite and the culture are intimitaly mixed in the porportion of 7 cubic centimetres of culture to one cubic-centimetre of sodium nitrite solution. The mixture is stratified with dilute sulphuric acid. Where the two layers join, a red-brown ring will form, which is the indol test. If the heat of reaction is great, the ring will form a bit further up in the solution. Since the color is practically limited to a narrow zone, a more concentrated solution of indol will obtain than if it were diffused throughout the whole amount of culture. Concentration of indol means a concentration of color, and the test is thereby rendered more delicate. Of course the test not only should be made for indol, but for nitroso-indol as well. This reaction will give positive or negative readings in less than an hour, and the writer has found it to be eminently satisfactory.

Precautions and sources of error in making the Indol Test.

Great variations are found to occur in different samples of the same brand of peptone; one sample will give excellent results, while another sample will be found to be unsatisfactory.

One sample of peptone that I used in this investigation gave most excellent results with the indol test; the pyocyaneus and typhoid cultures gave decided reactions, while ordinary peptones give at best traces of indol with the same organisms. Peckham (10) has found that _Bacillus typhosus_ will form peptone if a peptone made by pancreatic digestion is employed; the sample of peptone referred to above must have had the same substances that Dr. Peckham's peptone contained.

The practice of heating the culture and the acid is open to one serious objection: the acid seems to cause, even in control tubes, an artificial formation of the nitroso-indol reaction at times; the culture in which the organism is grown may at the same time show no signs of indol. Concentrated sulphuric acid will produce the same effect, and one should use dilute acid.

Commercial sulphuric, nitric and hydrochloric acids are found to contain traces of nitrite, hence only chemically pure acids should be employed in determinations of indol. Certain bacteria not only reduce nitrate to nitrite, but carry the process on to the free ammonia stage, or even to free nitrogen. Hence, if one makes a test for nitroso-

indol after the cultures have been incubated for a week or more, one is apt to find that no reaction is given, whereas if one tested the same solution earlier, a very distinct reaction would have taken place. Many of the Bacillus coli cultures tested gave the same result, and two tests had to be made, one at the end of the second day, the other at the end of the tenth day.

Summary;

In order to obtain the best and most constant results in testing for indol, one must use chemically pure acid; not more than two drops of concentrated sulphuric acid should be used for each cubic centimetre of culture to be tested. The acid should be stratified, not mixed as is the ordinary proceedure.

Final observations should be made at the end of an hour and a half, and the reactions carried out at the room temperature.

Control tubes, which have been incubated with the cultures should be tested for indol to insure its absence in the uninoculated tubes.

Reduction of Nitrites.

Many bacteria have the power of reducing nitrates to

nitrites, to free ammonia, and even to free nitrogen. In actual practice, tests for nitrite and free ammonia only are made.

The solution in which the reducing power of bacteria is to be tried consists of one gram peptone, two tenths of a gram of chemically pure sodium (or potassium) nitrate, made up in one litre of nitrite free water.

Since nitrites and free ammonia (for this solution answers equally well for the ammonia test) may be absorbed from the air, one must always incubate at least four controls with the cultures to be tested. Four tubes, two for the nitrite test and two for the ammonia test, are taken, because the test is a very delicate one, and one must take the average of the controls to insure accuracy of interpreting the results.

The amount of nitrate is determined in the usual way; by adding sulphanilic acid and alpha naphthylene hydrochlorate to each tube, one cubic centimetre of each solution. The depth of color is porportional to the amount of color present. If one wishes to make the test quantitative one may make a set of standards, and after sub-

tracting the amount of nitrite in the controls, determine by comparing the depth of color, the excess in the culture tubes. The quantitative determinations are of no value whatever for cultural work; they are extremely variable, with different cultures of the same species, and with the same culture at different times. In fact, practically no quantitative determinations of bacterial activity are of value.

With certain cultures one gets such an excessive formation of nitrite that an actual precipitate forms; in one case, the color changed after standing from the usual red to a pale yellow; I found that by diluting such samples 1/25 with nitrite free water that this phenomenon ceased.

Free Ammonia Production.

The same solution that one uses in the nitrite reduction test will also be suitable so far as composition is concerned for the free ammonia test; one incubates controls as before also. The test for free ammonia is carried out by adding one cubic centimetre of Nessler solution to both the culture and control tubes. Any excess of yello w color in the culture tube indicates the pres-

ence of free ammonia. The same remarks apply to the quantitative determination of free ammonia as to nitrite reduction.

Phenol production.

Phenol determinations are made by some bacteriologists as a routine practice, but the determination is a rather difficult one, and requires special media and distillation so ordinarily one does not attempt this test. There are, however, certain points involved in the actual production of phenol by bacterial action which illustrate the character of bacterial decomposition, and the production of phenol from tyrosin,(the probable source of phenol) will be chosen as an example.

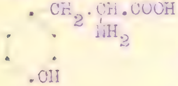
Gelatin contains no tyrosin, and one would expect a priori to obtain no phenol in the decomposition of gelatin. Such in fact is the case.

It will be remembered that bacteria seem to act by splitting off amido nitrogen as free ammonia, the oxidization of methyl groups to carboxyl groups, and finally by splitting off the carboxyl groups as carbon dioxide.

One can readily obtain tyrosin by pancreatic digestion of certain proteid, and as bacteria have been shown to pro-

duce tyrosin, we will start with this compound and show the successive steps whereby phenol is produced.

Tyrosin is para-oxyphenyl-amidopropionic acid.



the amido group spltis off, forming free ammonia and p-oxyphenyl-propionic acid.

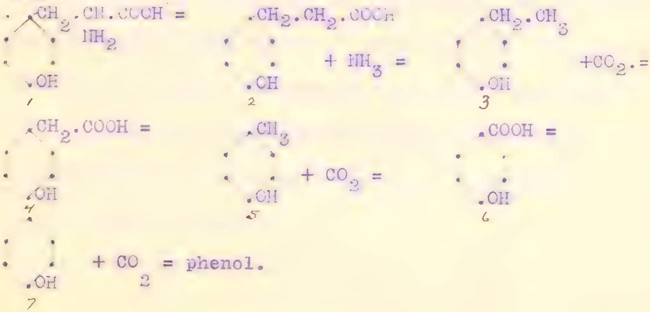
The carboxyl group splits off as carbon dioxide next.

This leaves p-oxyphenyl-acetic acid, after the methyl group has been oxidized to a carboxyl group.

Next the carboxyl group splits off, forming as before carbon dioxide, and paracresol.

The methyl group is oxidized to a carboxyl group, and removed as carbonic acid, and we have phenol left.

The following table will illustrate this action, step by step.



A Method of Tabulation of the Characteristics of Bacteria.

(see a preliminary report upon this subject by the writer, Proceedings of the Thirtieth Annual Meeting, American Public Health Association, Dec. 1902).

Everyone must be aware of the fact that if one wishes to compare a series of data, the descriptions will be more readily comprehended, similitudes and dissimilations will be more apparent, if the quantities to be compared are expressed by numerical symbols rather than by written expressions. If one can express (symbolically) by numerals the possible variations in growth forms of bacteria upon the different cultural media, and if these numerical symbols are arranged in logical sequence according to the different media, one has at once a means of rapid comparison of descriptions of two or more bacterial species. All that is necessary is to arrange the data to be compared so that like characteristics shall be placed under like characteristics; the identity or lack of identity will at once become apparent.

In order to make such a system valuable for general

bacteriological work, one should determine all the possible variations of growth forms occurring with all the media employed in bacteriological research. To these variations numerals are assigned; if now, the particular growth forms characteristic of a particular bacterium be arranged in proper order under the media upon which such descriptions are made, employing in this connection the numerical equivalents representing the growth forms, one can represent numerically (decimally) the description of this organism.

The terms representing the various cultural reactions must confirm to certain well defined biological principles; they must be general enough to cover the slight variations to which all living things are prone; specific enough to differentiate the particular characteristic from all other characteristics.

If to terms fulfilling these conditions, one assigns numeral equivalents, such numerals, properly chosen and arranged will indicate, in a very abbreviated, concise form, the reactions of any bacterium upon the different media, quite as well as a written description and have the great

advantage of facilitating comparisons with other organism similarly arranged.

In working out by experiment a series of terms fulfilling the conditions noted above, not only must the variations occurring as a result of slight differences of growth form, differences which are of intensity rather than of kind, be taken into consideration, but one must also regard the 'personal factor' of individual interpretation of these characteristics. For example, an observer might regard a given growth upon an agar slant as filiform, while another investigator would designate it as an echinate growth; either interpretation would be equally correct.

We have then, two sets of descriptions involved in cultural descriptions: one, slight variations in the growth forms themselves, the other slight differences of opinion as to the proper designation of the growth by different observers. The latter fact is the 'personal equation' mentioned above; bacteriologists up to the present time have paid no attention to this very important point, although scientists engaged in measurements of precision have recognized and applied it to their work,

astronomers have gone so far as to have this personal equation worked out and mathematically expressed for certain observations.

The fact that these variations do occur is not to be interpreted as indicating anything more serious than another expression of the biological principle of variation.

In this dissertation, attention has been paid to this very important factor: the actual demonstration that there could be, and actually are, personal variations was demonstrated in the following manner. A series of organisms were grown upon the customary laboratory media. A number of students made individual, independent observations of these cultural phenomena and recorded their results. At the same time the writer made a similar series of observations and recorded them in a similar manner. A series of tables will be found appended in which the results of these observations are expressed in the numerical way. In general three students made observations upon a given set of cultures; I made my observations, and recorded them in red ink so that one could easily distinguish between the different sets.

The value of the numerical system of recording the cultural characteristics of bacteria will be seen by an examination of the chart. One can see by glancing over a table the similarities and variations in the descriptions of bacteria; identity of description (numerals) means identity of characteristics, and conversely. A final advantage lies in the fact that the descriptions are orderly, arranged in a definite, logical sequence, and that incomplete descriptions cannot be obscured by lengthy descriptions of unimportant points. On the other hand, an incomplete description is painfully evident.

The tables appended to Part 1 are the tabulated results of study of a few species of bacteria, made by students beginning the subject of bacteriology.

The tables have decided interest from the fact that they demonstrate the 'personal factor', referred to above, is not an hypothetical possibility, but an actual reality in bacteriological investigations. Although perhaps this is the first absolute demonstration of this fact, one has but to tabulate in like manner the descriptions of bacteria to show that many bacteria, from their descript-

ions at least, differ from one another merely by some insignificant detail: such slight variations are not only of no importance specifically, but and not of sufficient value to define 'varieties' of bacteria.

When one considers that a certain school of bacteriologists rely almost exclusively upon cultural characteristics as criteria for division of bacteria into species, the insufficiency of much of the past work, and the need of systematic, complete study of all bacterial species becomes very apparent.

No attempt has been made in this connection to draw conclusions as to the percentage accuracy of certain cultural and biochemical reactions employed at the present time for bacteriological diagnosis. All that one can logically do with the data presented herewith is to derive information concerning the personal factor in interpreting the same phenomena by different individuals.

It should be stated in conclusion that while the system appears complicated, it should be borne in mind that the facts to be explained are complicated; that students, beginners in bacteriology had no apparent difficulty

in making and recording correctly their observations. In general, even from the start, the students could make the necessary observations more quickly than corresponding observations necessitating the use of written descriptions; they learned to look for essential features, and to express the same clearly, orderly, logically and concisely. They successfully avoided unduly emphasizing unimportant features, and gave the necessary attention to important details.

At the close of Part 1 will be found a legend giving the standard terms for use in bacteriological descriptions together with their numerical equivalents. By referring from the tables to the legend, one may interpret any symbol into its equivalent standard term. These terms are modified from the excellent work of Chester, Manual of Determinative Bacteriology, and the writer takes this opportunity to thank Professor Chester for his kindness in his friendly criticisms of the work.

Method for the Decimal or Numerical System of Recording the Cultural Characteristics of Bacteria.

Legend.

Slant cultures; agar, serum, potato.

Form of Growth.

1. Filiform; uniform growth, without special characters.
2; Nodose; consisting of rather large, discrete colonies.
3; Beaded; consisting of small, closely placed colonies.
4; Echinate; beset with acicular extensions.
5; Villous; beset with short, undivided hair-like extensions.
6; Plumose; a delicate, feathery growth.
7; Arborescent; tree-like: with branched, hair-like extensions.
8; Grows only in the condensation water (on agar).
9; Spreading; grows over the whole slanted surface.

Elevation of Growth.

1. Flat; thin, spreading over the surface to a moderate degree.

2; Effused; more delicate and spreading than the pre-
ceding.

3; Raised; growth thick, with abrupt terraced edges.

4; Convex; surface the segment of a circle.

5; Capitate; more convex than the preceding: hemi-
spherical.

6; Umbilicate; depressed in the centre like a navel.

7; Umbonate; having a boss or elevation in the middle.

Topography of Surface Growth.

1; Smooth;surface even without any of the following
distinctive characters.

2; Alveolate; marked by depressions separated by thin
walls, like a honey comb.

3; Punctate; dotted with minute punctures, like pin-
pricks.

4; Bullate; covered with blister-like prominences.

5; Squamose; covered with scales.

6; Papillate; beset with papillae, or mamma-like
processes.

7; Rugose; short, irregular folds.

8; Contoured; an irregular, but smoothly undulating

surface like the surface of a relief map.

9; Rimmose; abounding in clefts or cracks.

Optical Characters.

10; Transparent, extremely delicate film.

11; Vitreous; transparent and colorless.

12; Oleaginous; transparent and yellow, olive or linseed oil colored.

13: Resinous; transparent and brown, varnish or resin colored.

20. Translucent; translucent and colorless.

21; Paraffinous; translucent and white.

22; Opalescent; translucent, grayish-white by reflected light, smoky-brown by transmitted light.

23; Nacreous; translucent, grayish-white with pearly lustre.

24; Sebaceous; translucent, yellowish or grayish white.

25; Butyrous; translucent and yellow.

26; Ceraceous; translucent and wax colored.

30; Opaque, no optical characters other than opacity.

Lustre of Growth.

1. Shining.

2- Dull, without lustre.

3- Waxy; lustre like paraffin.

4; Iridescent; metallic lustre.

Stab Cultures, gelatin or agar.

Non-Liquefying Line Growth.

1. Filiform; uniform growth, without special characters.

2; Nodose; consisting of rather large, discrete colonies.

3; Beaded; consisting or rather closely placed colonies, small in size.

4; Echinate; beset with acicular extensions.

5; Villous; beset with short, undivided, hair-like extensions.

6; Plumose; a delicate, feathery growth.

7; Arborescent; branched, tree-like: with branched, tree-like extensions.

Liquefying, Line Growth, Form of Liquefaction.

1; Crateriform; a saucer-shaped liquefaction.

2; Saccate; shape of an elongated sack, tubular or cylindrical.

3; Infundibuliform; shaped like a funnel, conical.

4; Napiform; shape of a turnip.

5; Stratiform; liquefaction extending to the walls of the tube, and downward horizontally.

6; The liquefied portion dries up as fast as formed. Usually manifested as a slight sinking of the surface growth.

Surface Growth, non-liquefying cultures. (Also Plate Colonies) See Elevation, Slant Cultures.

Plate Colonies, Agar and Gelatin.

Form of Colony.

1; Round.

2; Fusiform; elliptical, tapering at each end.

3; Conglomerate; an aggregation of similar colonies.

4; Cochleate; spiral or twisted like a snail shell.

5; Amoeboid; very irregular, streaming.

6; Myceloid; a radiating structure like a mould.

7; Filamentous; an irregular mass of densely woven filaments.

8; Rhizoid; an irregular branched or root-like colony.

9; Rosulate; rosette shaped.

Detailed Characters of Surface. (See Topography of Slant Cultures.)

Surface Elevation of Colonies, (See Elevation, Slant Cultures.)

Internal Structure of Colony (Microscopic).

1; Homogeneous; structure uniform throughout.

2; Hyaline; clear and colorless.

3; Finely granular.

4; Areolate; divided into rather irregular or angular spaces by more or less definite boundaries.

5; Grumose; coarsely granular; particles in clustered grains.

6; Moruloid; having the appearance of a morula;divided into more or less regular spaces.

7; Clouded; having a pale ground with ill-defined patches of a deeper color.

8; Gyrose; marked by wavy lines.

9; Filamentous,Floccose; having a filamentous or dense felt-like structure.

Edge of Colony.

1; Entire; without toothing or division.

2; Waved; slightly undulating.

3; Lobed; consisting of lobes.

4; Serrated; notched on the edge like a saw.

5; Auriculate; with ear-like lobes.

6; Ciliate; hair-like extensions, radially placed.

7; Erose; as if gnawed, irregularly toothed.

Optical Characters; (See Optical Characters, Slant Cultures)

Biochemical Features.

Bouillon. Pellicle, Turbidity and Sediment.

Pellicle;

1; o pellicle, ring formed.

2; Pellicle membranous.

3; Pellicle tenacious (tough)

4; Pellicle flocculent.

5; Pellicle granular.

6; Pellicle wrinkled, thin.

7; Pellicle thick, wrinkled.

Sediment.

1; Sediment flocculent.

2; Sediment granular.

3; Sediment viscous.

4; Sediment compact, amorphous.

Turbidity.

10. Turbidity slight, clears up on standing.

11; Turbidity slight, non-characteristic.

12; Turbidity slight, flocculent.

13; Turbidity slight, granular.

14; Turbidity slight, viscous.

20; Turbidity decided, clears up on standing.

21; Turbidity decided, non-characteristic.

22; Turbidity decided, flocculent.

23; Turbidity decided, granular.

24; Turbidity decided, viscous.

Milk Cultures.

Coagulation.

1; Coagulates only on boiling.

2; Coagulation in the cold, coagulum hard, flocculent.

3; Coagulation in the cold, coagulum hard, compact.

4; Coagulation in the cold, coagulum hard, granular.

5; Coagulation in the cold, coagulum soft, flocculent.

6; Coagulation in the cold, coagulum soft, compact.

7; Coagulation in the cold, coagulum soft, granular.

8; Digestion without a coagulum being formed.

Liquefaction of Casein.

1; Liquefaction slight, whey turbid.

2; Liquefaction slight, whey clear.

3; Liquefaction decided, whey turbid.

4; Liquefaction decided, whey clear.

5; Liquefaction complete, whey turbid.

6; Liquefaction complete, whey clear.

Chromogenesis and Fluorescence.

Before discussing Chromogenesis and Fluorescence, it will be well to define exactly what we mean by these terms when applied to Bacteriology.

Chromogenesis; the ability of certain bacteria to produce among other products of metabolism, certain colored substances is called chromogenesis.

Fluorescence, strictly speaking, is the property possessed by certain substances of converting obscure rays, principally actinic in character, into luminous rays. The term, however, is applied differently by different bacteriologists; some merely mean the production of a pigment which is soluble in agar; some define fluorescence as a property in virtue of which certain soluble pigments

which exhibit complementary colors as they are viewed by transmitted and reflected light respectively. In the present dissertation, however, the term will be used to designate any soluble pigment; in fact the term soluble pigment would in this connection, be more descriptive. Many bacteria produce a soluble pigment in addition to the regular pigment, while very few produce a true fluorescent pigment.

Method of recording chromogenesis and fluorescence. Before outlining the method, it should be said at the very start that chromogenesis should always be determined by viewing the <u>surface</u> of the agar slant; reflected light should always be used. Under these conditions, one sees the actual bacterial growth, while the observations are not vitiated by the effect of a layer of more or less highly colored medium. Observations of chromogenesis for classification purposes should be made upon slant agar.

The pigments produced by bacteria are extremely varied; colors that are not spectral, e.g. brown, are frequently produced, and mixtures of colors that are hardly describable may occur.

The pigments produced are not pure, that is, they are so to speak not chromatic 'alloys' but are mixtures, such as one would obtain if one mixed colored particles in varying perportions; precisely as the predominating color with such colors might be difficult, so in the bacterial pigments, the predominating color is often difficult to distinguish.

This often leads to confusion among bacteriologists; one observer may interpret a mixture of colors containing (say) blue and red as a blue-red; the blue seems to him to predominate, while another would call the same red-blue, with the red predominating.

One must consider these points if one wishes to make a system which will be at all satisfactory for color determination.

I have done it in the following manner; to begin with, bacterial pigments are usually mixtures of not more than two colors. Any error of interpretation must be in the determination of the predominating color. If we represent symbolically, by the decimal system, the possible colors

produced by bacteria, and so tabulate them that two observers, each of whom will differ as to the most prominent color will be able by a simple process to translate the color at will from one predominating color to the other. For example, let '7' represent blue, and '3' represent red. (see the legend under chromogenesis). If observer A calls a particular growth red-blue, he would indicate that as '37' in the table. Observer B, calling the same thing blue-red, would write 73, the reverse of Observer A.

This is the basis of the proposed system. All the colors, except white, brown and black, which are colors in the bacteriological sense, are arranged in the order in which they occur in the solar spectrum.

The list becomes, then, 1-white; 2-brown; 3- red: 4-orange; 5-yellow; 6-green; 7-blue; 8-black. 9 is reserved for fluorescence.

The appended numbers will alwa ys indicate, under chromogenesis, something white, 3 something red, etc.

31 would indicate a mixture of red and white, red predominating; 74 would indicate blue-orange, the blue predom-

inating. With this explanation, one will be able to make use of the following table. The arrangement, and in fact, the whole conception of chromogenesis is not correct scientifically, but it is the best that can be done with the end in view; of making a system whereby one can represent in a simple manner, the chromogenic properties of bacteria. The pure colors, white, brown, red, etc. are represented with a '0' as the second figure; all combinations with the pure color are represented with the initial number of the predominating color first, then the color mixed with the predominating color.

10. white.	20. brown.
11. gray.	21. brownish.
12. white-brown.	22. light brown.
13. white-red.	23. brown-red.
14. white-orange.	24. brown orange.
15. white-yellow.	25. brown-yellow.
16. white green.	26. brown-green.
17. white-blue.	27- brown-blue.
white-black; slate.color.	28- brown-black.

30. red.

31. reddish.

32. red-brown.

33. light red.

34. red-orange.

35. red-yellow.

36. red-green.

37. red-blue. (purple)

38. red-black;dark red.

40. orange.

41. orange-white.

42. orange-brown.

43. orange-red.

44. light orange.

45. orange-yellow.

46. orange-green.

47. orange-blue.

48. orange-black;dark orange.

50. yellow.

51. yellowish.

52. yellow-brown.

53. yellow-red.

54. yellow-orange.

55. light yellow.

56. yellow-green.

57. yellow-blue.

58. yellow-black;dark yellow

60. green.

61. greenish.

62. green-brown.

63. green-red.

64. green-orange.

65. green-yellow.

66. light green.

67. green-blue.

68. green-black;dark green.

70. blue.

71. bluish.

72. blue-brown.

73. blue-red (violet)

74. blue-orange.

75. blue-yellow.

76. blue-green.

77. light blue.

78. blue-black;dark blue.

80. black. *

Fluorescence or soluble pigment.

91 pigment opalescent,whitish.

92. pigment brownish.

93. pigment reddish.

94. pigment orange colored.

75. pigment yellowish.

96. pigment greenish.

97. pigment bluish.

98. pigment very dark colored.

* black is described as that constituent that produces a darkening of a 'pure color'; see 9 after each of the promary colors, 19, 49,etc.

Part 2.

Description of the Morphological, Cultural and Biochemical Characteristics of Certain Bacteria from Different Sources upon the Same Lot of Media.

There seems to be a tacit understanding among bacteriologists that a certain more or less definite complex of characteristics, produced by a given organism upon certain media is a good and sufficient specific definition of that organism; that these reactions will always distinguish it from all other kinds of organisms, and that one may be certain of the identity of a freshly isolated or unknown species having these characteristics in common with the type.

In other words the above mentioned attributes will distinguish a series of bacteria which resemble each other more than they resemble anything else as the same species, and will always serve to separate such organisms from all other organisms.

It is of fundamental importance to define as sharply as possible the criteria for such divisions of bacteria into families, genera and species for the purposes of classification.

A priori, one would expect in a subject so important to bacteriology, whose literature contains the names of hundreds, even thousands of 'species', that the classification of bacteria at least would be permanently fixed. At least one would there should be some sort of agreement concerning the fundamental division of bacteria into genera.

Even a casual perusal of the literature will show that such is not the case; that even the limits of the Bacteriaceae are not sharply defined.

Bacteriologists speak with certitude of Bacillus coli for example, yet if one asks different individuals to define this particular species of bacillus, the answers are wonderfully varied.

A few examples will suffice.

1; Bacillus coli is an organism which does not liquefy gelatin, which ferments dextrose with the production of gas, reduces nitrate to nitrite, coagulates milk and produces indol.

2; Bacillus coli is a motile bacillus, fermenting both dextrose and lactose.

3; Bacillus coli a motile bacillus fermenting dextrose

with a gas formula (gas ratio) of $H/CO_2 = 2/1$.

4; Bacillus coli an organism producing 50 % or more gas in lactose.

Many more examples could be cited, but enough has been presented to indicate the lack of uniformity in this respect.

One may object, and object rightly that these are merely methods for the routine identification of the organism in question: that they are not labored descriptions for purposes of classification.

This does not, however, detract from the force of the argument. Observer A, because of long experience believes he can detect and identify absolutely without question Bacillus coli as an organism different from all other organisms because it produces more than 50 % gas in lactose. Observer B, comparatively inexperienced, using precisely the same method as recommended by A may fall into serious error.

Realizing the great differences in the method of identification of bacteria, and the varied criteria for their isolation and determination, I have obtained cultures of a few of the very common organisms from different

sources and have studied them upon the different media used for ordinary bacteriological research under the same conditions in order to compare their various reactions.

If organisms representing different strains of the same species are compared by the same observer under the same conditions, one may compare the descriptions and say definitely that such bacteria do or do not agree in essential characteristics.

If the descriptions do agree, provided the criteria established are sufficiently complete to include those characteristics considered necessary and sufficient for species determination, one may assume that in spite of the apparent differences in method referred to above, the final diagnosis was correct in each case; that the different, variable criteria used by different observers represent merely different points of view of the same object.

If the descriptions do not agree in their essential points, we are either at fault concerning our choice of characteristics, or else we are not dealing with the same

organisms.

The organisms studied to determine the correctness of the above points were several strains of Bacillus coli, typhosus, prodigiosus and pyocyaneus.

These organisms are found in almost every laboratory and represent almost every variation in morphological, cultural and biochemical features. Hence they lend themselves admirably to a research of this sort.

Method of Study.

The general method of preliminary cultivation, method of tabulation of results, preparation of media, inoculation, and other precautions have already been discussed in detail (see Part 1.)

The cultures of each organism, as soon as received, were plated out and given preliminary cultivation in the usual way.

After they had their preliminary cultivation, and were in good active vegetative condition, multiplying rapidly, various media were seeded, using the usual precautions, stored in the thermostat (except gelatin cultures) and removed for observation upon the first, third, seventh and tenth days. Records of their appearance upon all the

cultural media were made upon the third, seventh and tenth days.

Biochemical and Cultural Characteristics of *Bacillus coli*.

In all, fourteen cultures of the colon bacillus were received in response to the letters sent to different laboratories. As soon as these cultures arrived, they were plated out and found to be pure. Accordingly, after the usual preliminary cultivation, they were seeded upon the customary media.

The standard agar, agar number 4. was the one chosen upon which descriptions of the agar slant and agar plate. Gelatin stab, gelatin plates, nitrite solution, indol (Dunham's) solution, milk, potato, blood serum and finally fermentation solutions of dextrose, lactose and saccharose were also made.

Table number 1 shows the results of the ten day observations of growth of these organisms.

Not only were the Cultural and Biochemical results recorded, but an attempt was made to note the relative luxuriance of growth according to the method of Fischer, (loc. cit. p. 55).

Fischer designates by + a slight growth, ++ a decided

growth and +++ a luxuriant growth. The results were interesting in that the growth upon agar number 4 was the most luxuriant;(other samples of agar were used,as will be described in Part 3.) Agar 4, it will be remembered, was made according to the recommendations of the American Public Health Association Committee Report. However, for descriptive purposes, the results of the quantitative determinations were of minimal importance,and are omitted from the descriptions. Nevertheless, slight and constant differences were observed which seemed to consistently hold true for all the different cultures examined. This observations agrees well with similar observations made in New Orleans by the writer and Weston, (see Some Common Bacteria in American Streams, Weston and Kendall; American Public Health Association, Proceedings, 1901.)

The colonies developing upon agar and gelatin plates were the source of considerable difficulty in that there was considerable variation in their general form. At the same time, the majority of the colonies presented a typical picture agreeing well with the classical descriptions of the organisms in question.

In describing colonies, the Form, Elevation, Topography, Internal Structure, Optical Characters and Chromogenesis were observed upon the gelatin plates.

This corresponds well with the physical attributes which one would consider necessary and sufficient for a description of a solid in space; its Form, Structure and Color.

Upon agar plates the Chromogenesis and Optical Characters were omitted, because they were found to be the same as the same characteristics obtaining upon the agar slant.

With the exception of the Internal Structure, all observations were made macroscopically; this is logical procedure because the microscopic characters are they upon which one would rely for the differentiation of one species from another in a plate containing a mixed culture. In such a case one would almost invariably rely upon macroscopic rather than microscopic differences.

The question of variations from the type was dealt with in the same general manner; typical colonies, when they were in the majority were selected for description precisely as one would pick naturally a typical colony

for isolation, so I picked a typical colony for description.

The uniformity of description then, obtaining throughout the descriptions of the colonies is due to the fact that they represent average or mean appearances which the organisms may be expected to present in a general study of the subject.

The writer considers that not only should the mean appearance of the colonies should be described, but also that there should be a definite maximal and minimal limit to the number of colonies existing upon the plates at the time the descriptions are made; too many colonies will cause an antagonism, a struggle for existence, which will inhibit the general welfare of the organisms, resulting in too small colonies and a mutual inhibition of growth. Too few colonies will lead to error because there may be intrusion of outside germs; these may or may not be easily recognized as contaminations.

If there were more than 75 or less than 6 colonies on a plate, it was discarded and a fresh plate made.

The morphology of the fourteen colon colonies described, representing an average colony of each strain, was

remarkably constant; except for variations in size,noted elsewhere,there was practically no difference.

The morphology will be described in detail in Part 4.

All the cultures were facultative anaerobes,growing at 20°, 37°, possessing no demonstrable spores,and not staining by Gram's method.

Cultural and Biochemical characters in detail.

Agar slant; three general forms of growth could be demonstrated, namely villous, spreading and filiform. These differences were quite evenly divided between the various strains as may be seen by an examination of Table 1. The variations are not to be interpreted as indicating anything more fundamental than reactions to slight changes in the relation between luxuriance of growth,and local conditions upon the surface of the agar. A slight excess of moisture, unsteadiness of making the inoculation and varying amounts of culture added to a slanted surface will amply explain such differences, which are differences in the extent of growth, not variations in the mechanics of reproduction upon the part of the organisms. If on the other hand one culture should always give a slight, fil-

iform growth while another gave a more or less pronounced arborescent growth, one would be justified in seeking a further explanation of these phenomena. In all probability the latter variations would indicate changes more profound than the ordinary variations occurring in living things due to such the changes in environment acting for a short time.

However unimportant they may seem, one should always note such deviations of growth in the accurate and final descriptions of each organism, when such description is published for the first time, indicating clearly the variations observed upon each medium at different times, and under different conditions; such a proceeding would effectually forestall a claim by a future writer of a new species based upon the strength of trifling changes in growth form. This may at first seem superfluous and unnecessary, but a detailed study of published descriptions of bacteria will reveal simply astonishing examples of negligence in this respect.

The Topography, Elevation, Lustre and Chromogenesis of the slant cultures upon agar of the Bacillus coli were within the limits of a rational description, quite constant.

Gelatin stab cultures; all cultures, except those of Culture number 9, did not liquefy gelatin. The latter culture seems to be *Bacillus cloacae*, Jordan.

This culture, of all the cultures received, was the only one which proved to be other than was stated upon the label. It furnishes an interesting example of the confusion attending the identification of *Bacillus coli*; if one is content to call an organism which ferments dextrose with 50 % gas production, reduces nitrate, forms indol, and is a motile organism, this culture, number 9, is a typical example of the colon bacillus. A careful study of similar cultures will reveal possibly other examples of a similar nature.

The line growth of the gelatin stab cultures showed great variations; this is in accord with the observations of many bacteriologists, and is specifically demonstrated in Whipple's exhaustive study of gelatin, (loc. cit.) It emphasizes the fact that ordinarily the line growth in gelatin stabs is quite unsuitable for the identification of bacterial species.

Bouillon; the formation of a pellicle in about a third of the cultures of the colon bacillus, and the ab-

sence of a membrane in the other two thirds of the organisms is not surprising; the writer has noticed repeatedly the appearance of a pellicle at one time and the absence of the same at another time, even in descendants of the same organisms.

A very important factor in determining the formation of a ring or a pellicle in many instances is the diameter of the tube in which the culture is grown; tubes having a diameter of from 12 to 25 millimetres will often show a ring formed at the surface of the liquid, while a parallel culture will form a delicate film if the diameter be less than 12 millimetres. The explanation may be either that the narrower tube contains less fluid, and consequently the nutriment is used up, except at the surface where oxygen and consequent fresher material is present, or that the tenacity of the membrane is not enough to bridge over a gap of more than a few millimetres.

The turbidity and sediment were quite constant in all the cultures studied, and need no further mention here.

The relation between the relative nutritive value

of the different samples of bouillon and the luxuriance of growth seems to be the factor which determines the amount and to a certain extent the character of the turbidity and sediment produced by bacteria grown in bouillon.

Blood serum; the chief value of blood serum is to furnish a medium which more or less closely approximates the composition of the fluids of the body. This is not without importance in the study of certain organisms which do not grow well upon ordinary media; media which differ radically in composition from those of the host upon which they are normally found. The value for general diagnostic work, however, is not great. The only characteristic of importance is the possibility of liquefaction, and this is usually paralled by the liquefaction of gelatin. Of course one can determine the liquefaction of blood serum at the body temperature, while gelatin cultures usually are kept at room temperature.

Potato; the cultures investigated grew luxuriantly, and upon the whole fairly constantly upon potato. There were certain slight differences, both in the form of growth and the relative luxuriance of growth, but the differences

were comparable in every particular to those upon the agar slants, and have of course the same significance.

The uniformity of growth is due for the most part to the fact that the potato slants were made from the same lot of potatoes. If a different lot of potato had been used, the results would again have been constant upon the new lot, but would show slight, more or less constant differences from the growths upon the old lot.

The remarkable similarity in the mean appearance of the colonies of the colon bacillus upon agar and gelatin plates has already been commented upon; the average appearance of the colonies has been made the basis for the descriptions in the tables; it will be remembered that such descriptions are valuable because they are the characteristics upon which one would rely if one were isolating the organisms from a mixed culture.

Milk; Culture number 9 was the only one of this series which did not coagulate milk and produce acid. All the other cultures agreed in these particulars, and in addition, many showed a tendency to decolorize the litmus. It is a question whether the coagulation of the casein was due to a rennin-like enzyme formed and excreted by

the bacteria, or whether the coagulation was due to the production of a certain amount of acid.

The decolorization of the litmus might also be explained by the production of an excess of acid, or by a strong reducing action on the part of the organisms, changing the blue litmus to the colorless, or leuco dye. It seems probable that the latter hypothesis is the correct one.

Fermentation reactions; about one fifth of the cultures examined fermented all three sugars; dextrose, lactose and saccharose. This agrees very will with the general experience of bacteriologists that a few colon cultures will produce gas in all three of the ordinary sugars, while the larger percent will ferment only dextrose and lactose, not the saccharose.

Culture number 9 seems to be one of the Cloaca group which agree with the Colon group in this respect. The majority of the Colon and the Cloaca bacilli do not ferment saccharose; yet there are some cultures which will produce gas in the saccharose.

Nitrate reduction and Free Ammonia production.

Only one culture did not produce free ammonia. All the cultures reduced nitrate to nitrite. The organism

which did not reduce the nitrite to free ammonia, culture number 8, formed nitroso-indol readily. Yet repeated trials failed to give a free ammonia test. There is no logical explanation of the peculiarity.

Indol production; again culture number [illegible] failed to agree with the other organisms; this was the only culture in which indol could not be demonstrated. Many of the organisms produced nitroso-indol.

The precautions noted under Indol, Part 1, apply particularly to the cultures of <u>Bacillus coli</u>; this organism reduces readily, and carries the reduction to free ammonia as a rule, and rather rapidly. Unless one examines the cultures early for nitroso-indol, the test may be negative, owing to the further reduction of the nitrite to free ammonia. The test for nitroso-indol should be made before the final reduction has taken place: if one makes several inoculations into the indol solution, and examines one every two days, the production of nitroso-indol can be easily demonstrated.

Summary of Colon Cultures.

With one exception, Culture 9, all the cultures ob-

tained as _Bacillus coli_ were shown by subsequent study to be typical organisms. There were slight variations in the form and relative luxuriance of growth upon the cultural media; variations which imperceptible merged into one another, showing that there are no fundamental difference in any reactions of the cultures. These differences however, were marked enough to be very noticeable to one studying the material critically.

Observers who base their diagnoses of bacterial species upon such appearances and to whom a culture which differs in any respect culturally from the type is regarded as a distinct species, even if the organisms be isolated from the same source, would have no hesitation in pronouncing the extremes in variation as separate and distinct species, regardless of the fact that the biochemical reactions were the same, and that the cultural growth forms merged imperceptibly into one another.

Bacillus typhosus.

Typhoid bacilli in pure culture are comparatively easy to identify.

Bacillus typhosus has the property of so modifying the blood serum of a patient ill with typhoid fever that this serum will agglutinate and immobilize the typhoid bacilli. The reaction, in dilutions of not less than 1/50 seems to be quite specific for these organisms.

Accordingly, the cultures of the typhoid bacillus as soon as they were received, were plated out to insure their purity, and then tested with the serum of a patient ill of typhoid fever in the Johns Hopkins Hospital.

The serum was fresh and agglutinated in a typical manner a known culture of Bacillus typhosus.

Each and every culture sent to the laboratory to be used in this research agglutinated, 1/50, in less than an hour, using the microscopic rather than the macroscopic agglutination.

Methods of making the Serum Reaction.

There are two microscopical method of observing the serum reaction with different organisms; one, the 'end-to-end' reaction, in which the organisms, usually not

more than two or three, remain with their ends in juxta-position for a longer or shorter time: the other, the so-called 'clump' reaction in which definite masses of bacteria remain agglutinated while the spaces between the clumps are practically free from bacteria. At the same time there is usually loss of motility upon the part of the bacilli, if the culture is motile at the start.

The former method is unsatisfactory; one cannot satisfactorily distinguish between 'agglutination' and the phenomenon which bacteria exhibit while growing slowly. In either case two or three organisms remain very near one another.

The clump or agglutination reaction on the other hand presents a very definite picture in positive cases; the organisms are gathered together in masses, while the field between the clumps is practically devoid of bacilli. In this work a careful microscopical examination of every emulsion was made at the time of preparation; this was to insure the absence of clumps before the characteristic agglutination on the part of the typhoid bacilli could take place.

Previous mention of the fact that all the cultures

agglutinated typically, and within the time limit. Culture number 3, of which more will be said later, agglutinated in less than five minutes.

The morphology of the typhoid bacilli will be discussed at length in Part 4. It is sufficient to mention here that the organisms were motile bacilli, not staining by Gram's method, growing well at 20°, 37° and facultative anaerobes.

Cultural and Biochemical Characteristics.

Agar slant cultures; while most of the cultures of Bacillus typhosus exhibited a filiform growth upon the agar slant, a few grew either as echinate or filiform growths. The latter forms are merely slightly exaggerated filiform growths and are to be interpreted as having the same significance in this connection as differences in size among individuals of the same species in the higher plants.

The remainder of the characteristics, Elevation, Lustre, Chromogenesis, Optical Characters and lack of Viscosity, were the same in all the cultures.

The variations noted above are rather less in extent than the corresponding observations upon Bacillus coli.

No logical deductions can be drawn from this fact, however; while the colon bacilli may have been derived from different sources, milk, water, sewage, etc, and while the typhoid bacilli must have come originally from typhoid cases, yet one is not justified in assuming that the variations are due to the differences in origin of the cultures. The facts do not bear such a supposition out either. There was no constancy in characteristics of growth form. At one time a culture would depart from the filiform growth and become spreading: again the same culture would be echinate. There is nothing hard and fast about the growth upon an agar slant. All that one can expect is that the variation will be consistent; an organism will be either filiform or some modification of a filiform growth, never arborescent or plumose for example.

Gelatin stab cultures.; none of the cultures received as Bacillus typhosus liquefied gelatin. On the other hand, they exhibited practically every known form of line growth; no one of the forms of line growth, however, could be said to predominate. A surface growth was present in about one half of the cultures examined, while the remainder showed no surface growth at all. The line growth and

the surface growth are of no diagnostic importance, although they are used by certain writers as criteria for division of bacteria into groups.

It is difficult to explain the presence of a surface growth in certain of the cultures and its absence in the remainder, because the organisms had precisely the same preliminary cultivation, they were all growing luxuriantly when inoculated, and were seeded into gelatin of the same lot. There was at the same time a vigorous line growth, which would argue against the explanation of this variation upon the ground of a lack of vitality with the cultures which were lacking in this respect. At the same time the phenomenon is not unusual.

Bouillon; pellicle formation. The results of the cultivation of the typhoid bacilli fails to agree with the majority of published descriptions of this organism in producing in many instances a distinct pellicle. A ring was formed in certain of the other cultures.

It should be stated in this connection that in almost every instance in which a culture has differed from the classical description of the species, the variations have been positive, that is, the culture did not fail in

any characteristic, but actually gave positive reactions in features that were supposed to be negative. This must be attributed to the preliminary cultivation in absence of a better explanation.

Turbidity and sediment; while there was nothing about either the sediment or turbidity which would distinguish _Bacillus typhosus_ from the majority of other bacilli, the cultures agreed very well among themselves in these characteristics. The broth cultures of the organisms were upon the whole more constant than were parallel cultures of the other bacteria studied so far.

Blood serum; none of the cultu res liquefied blood serum. The filiform growth predominated, and exhibited a tendency to spread slightly. This to a lesser extent occurred with _Bacillus coli_. Possible the reason may lie in the fact that serum is more nearly like the normal blood serum in composition, and furnishes a more favorable pabulum for the organisms, hence the increased luxuriance of growth.

No pigment was formed upon the serum.

Potato; with four exceptions, the typhoid bacilli did not grow visibly upon the particular medium employed

for these descriptions. This agrees very well with the description of the German writers who find as a rule that *Bacillus typhosus* does not grow visibly upon potato. These results are not constant, however; the same cultures inoculated upon other potato were the same in general character, but the cultures which previously grew visibly now did not show at all. On the other hand the other organisms grew visible, some at least.

The potatoes were not fresh; the cultures were made in the spring, while the other observations were made in the fall, with recently dug potatoes. The differences, then, may be explained partly upon this basis; it is a well known fact that tubers as well as many seeds have a latent period lasting from the time of ripening, in the fall into the spring. During this time they will not grow, but are gradually undergoing certain changes, possibly changes in reaction as well as enzyme change. Hence the difference in growth upon the spring and fall potatoes.

In no instance was the potato.discolored.

The colonies upon the gelatin and agar plates were quite uniform in appearance more so than the colon colon-

ies. The average appearance was chosen, as before, and this accounts for the similarity of the descriptions of the plate cultures.

Milk; Bacillus typhosus is said to produce three different reactions in milk: an acid reaction, which persists, an alkaline reaction which also persists and finally, an amphoteric reaction, that is, the initial reaction is acid, and after a few days usually not more than five, becomes definitely alkaline.

The cultures tabulated in the table, table 2, show two types of reaction, the alkaline and the acid type. The acid cultures were acid from the start, and remained acid, while the alkaline cultures were alkaline throughout the period of observation, ten days.

The amphoteric reaction did not occur.

Both litmus and plain milk was used, controls were incubated under the same conditions as the cultures, and the alkaline cultures retested, withthe same results each time.

This variation in reaction is not limited to Bacillus typhosus, Bacillus dysenteriae, (both the Shiga and Harris types) show the same cultural peculiarities, and in addit-

for two other variations, namely one in which there is the usual transient alkalinity, with a return to acid, then after a week or more the culture grows alkaline again, so there occurs two different alkaline reactions with a well marked acidity preceeding the last alkaline reaction. The other variation occurs when there is a transient alkalinity, then a faint acidity folowed after a week or more by a sudden increase in acidity, and no final return to the alkaline reaction. I have not noticed such reactions in the typhoid cultures, although the two organisms are quite similar in their cultural reactions.

Each of the five variations in the Morris and Shiga organisms will agglutinate with the same serum, and the same holds true for the variations in the typhoid cultures, all agglutinate with a specific typhoid serum.

These variations seem to be quite constant, and we are confronted with the problem of determining the value of such reactions for classification purposes. Do they indicate different species, different varieties, or they to be regarded as merely slight abnormalities. Such questions cannot be discussed in this connection, although they are of sufficient importance to mention.

indol production; one of the most interesting findings in this investigation was the production of indol by almost every one of the cultures of Bacillus typhosus.

Earlier descriptions of the typhoid bacillus agreed that this organism rarely or never produced indol. Peckham (loc. cit.) has found that this bacillus will produce indol if it is grown in media containing a specially prepared peptone, made by pancreatic digestion. In media containing this 'pancreatic' peptone, after a sort of preliminary cultivation, Bacillus typhosus produces indol in considerable amounts.

A sample of the so-called Witte's peptone was used in this research; it was noticed frequently that the cultures produced indol when grown in media containing this particular sample, whereas they would produce only small amounts, sometimes none at all, in other samples of the same make. This particular peptone must have been rich in substances from which bacteria can form indol. (tryptophane?)

Twenty out of twenty one cultures produced definite, clear cut indol reactions; if the same cultures were grown in one other sample of peptone, the only other sample

the different media showed certain variations;variations which were probably due, however, not to any lack of vitality upon the part of the organisms themselves, but to slight differences in the composition of the media, possibly to mechanical differences, as the distribution of moisture upon the surfaces of the cultures themselves.

Even the extreme variations were slight in comparison with the cultural variations of Bacillus coli. They agreed with the colon bacillus however, in the fact that the variations were always logical;variations in in extent, but not in character of growth. Upon the whole, the cultures of Bacillus typhosus were the most constant of any of the series of cultures studied in this work, and for this reason the most satisfactory.

tried, no indol was formed, or at least only an ill defined trace. Careful controls were made to insure the accuracy of the tests.

This at once raises the question of the value of the indol test in bacterial diagnosis. The best that one can do is to keep those samples of peptone which seem especially suited for indol production, and use them in all indol determinations. The test cannot be a very valuable one until bacteriologists can obtain samples of peptone that will be uniform in this respect. Mention of this point has already been made in Part 1; this is an experimental demonstration of the facts presented there.

Summary of the typhoid cultures.

Generally speaking, the Cultural and Morphological Characteristics of the various typhoid cultures agreed very well among themselves. There were, however, noticeable variations in a few instances; four cultures grew visibly upon potato, and four cultures produced an initial alkaline reaction in milk.

Furthermore, the relative luxuriance of growth upon

Bacillus prodigiosus;

With the study of Bacillus prodigiosus we begin the consideration of an entirely new and markedly different type of organism, the so-called chromogenic bacteria, or those forms of bacteria which produce pigment. The organism under discussion also liquefies gelatin, another characteristic which has not appeared up to the present time in this work.

As one reads the literature upon the chromogenic bacteria and compares the descriptions of these organisms with those of non-chromogenic bacteria, one becomes more and more impressed with the fact that bacteriologists have in general relied upon the color of the pigment, with possibly a few additional characteristics to identify this class of organisms. It is possible that such criteria are ample to identify chromogenic bacteria, and under these conditions one will expect to find the cultural reactions to agree in every particular, allowing the usual variations for mechanical conditions which we cannot control at present.

In response to requests sent to different laboratories

for cultures of *Bacillus prodigiosus* eight cultures were received. Of these only one was non-pigmented when it reached the laboratory; culture number 6: preliminary cultivation restored its pigment-producing properties, however.

Bacillus prodigiosus is interesting aside from its historical relations partly because it is closely allied to *Bacillus indicus*, an organism isolated from the stomach of an ape by Robert Koch.

With the exception of the fact that *prodigiosus* produces a scarlet color, and *indicus* produces a brick-red color upon slant agar, the two organisms are identical.

The pigment of these two organisms, particularly that of the former has been studied with great detail by both bacteriologists and chemists. Schneider has done the best work upon the chemistry. He finds that the pigment of *Bacillus prodigiosus* is a true dye; it will color permanently silk and other fabrics. The most interest, however, attaches to the reaction of the pigment of the organism to acid and alkalai.

If one adds to a solution of the pigment in alcohol, ir to a portion of a solid culture from the surface of

slant agar or potato acid, either acetic or hydrochloric, no change is apparent, or at most only a slight darkening. If one adds acid to a similar preparation of pigment obtained in like manner from Bacillus indicus, the color immediately, or mediately, turns from the brick-red to the scarlet or purple color of prodigiosus. If one adds alkalai to the prodigiosus culture, or to the acidified indicus culture, the color at once changes to the familiar brick-red characteristic of the latter organism.

This designation of scarlet and brick red applies to the color typical of these two organisms respectively. We shall see later that even descendants of the same bacterial cell, either prodigiosus or indicus, when placed upon media having the same composition, as nearly as we can tell, will produce at one time the scarlet color, at other times the brick-red color. The organisms in other words react to changes in environment which are beyond our powers of detection, and produce sometimes one, sometimes the other pigment, or some transitional shade between.

As will be seen by consulting Table 3, one may pass by imperceptible steps from one to the other color. In a few instances, the condensation water is scarlet, while the solid growth in the same tube is brick-red. In this case the transition occurs in the same culture. The whole question is a perplexing one, and it is indeed difficult to explain the occurrence of both pigments where the conditions both of gaseous and solid environment can hardly vary.

Cultural and Biochemical Characteristics.

Agar slant cultures; the chromogenesis has already been discussed quite fully. The form of growth was practically the same in all cultures; filiform or slightly echinate in appearance. The growth was luxuriant, shining, smooth and with one exception, devoid of viscosity.

The elevation in growth deserves mention; in general, the elevation furnishes data which enables us to form some idea as to relative luxuriance of growth between different cultures. If a culture one the one hand forms a hemispherical growth, and on the other hand, a second culture forms a flat growth, both filiform, one can imme-

diately declare the former to be a more luxuriant growth than the latter.

This characteristic, however, can be markedly changed by the method of preliminary cultivation; the cultures of Bacillus prodigiosus were at first rather sluggish, and produced only flat growths; a few rapid transfers to various media not only caused the growth to assume the more luxuriant, convex form, but there seemed to be a tendency to form pigment at a higher temperature. It is a well known fact that the organism as a rule does not form its characteristic pigment at 37°, but by a series of transfers from broth to agar, to potato, to broth again, I was able to produce pigment with all the cultures at 37°.

Gelatinstab cultures; all the cultures liquefied gelatin rapidly. Inasmuch as bacteriologists rely upon this characteristic as the first, and most important of the Biochemical characteristics of bacteria, this was very satisfactory. The form of liquefaction was, however, notoriously inconstant. There were hardly two forms of liquefaction alike. This emphasizes once more the fact that the gelatin stab culture is valuable only as a convenient method of determining the liquefaction or non-liquefaction of gelatin.

Broth cultures; about one-half the cultures formed rings in broth. The tendency toward ring formation varied slightly in the different cultures, although this is not wholly to be explained as an idiosyncrasy upon the part of these organisms. The diameter of the tube, as has already been pointed out in Part 1, has considerable influence in this respect.
For example, one of the cultures produced a ring in a tube of moderate diameter, (16 millimetres) while in a narrow tube, (10 millimetres) it almost invariably produced a thin but distinct membrane.
The lack of oxygen in the deep, narrow tube probably accounts for the transformation from a ring to a well-defined pellicle.

The turbidity and sediment were non-characteristic, except for their color; even this was not constant.

Blood serum; there was nothing particularly characteristic about the growth upon blood serum, either the form of growth, chromogenesis or relative luxuriance of growth. All the cultures, however, agreed in liquefying the medium with considerable rapidity.

Not all the cultures produced pigment, and those that di-

produce a colored growth were not characteristic; the color was merely a faint pink.

Potato; the variations in chromogenesis upon potato were quite as marked as upon slant agar. A very interesting point came to view in this respect; one would a priori expect that the variations in chromogenesis would be at least consistent; if a particular organism gave a brick-red upon agar, it should give a corresponding color upon potato. This was not the case, however. Some of the red cultures became scarlet, and vica versa.

Although the cultures were undoubtedly Bacillus prodigiosus in every instance, the indicus color appeared in some instances. In all other respects, the cultures were characteristic of prodigiosus.

A specific comparison of the agar and potato colors of the various cultures will indicate the changes obtaining as the organisms were grown upon the different media.

Culture 1. growth pink upon agar. scarlet upon potato.

* " 2. " brick-red " " red " "

" 3. " pink " " scarlet " "

" 4. " pink " " scarlet " "

" 5. " scarlet " " red " "

Culture 6. growth scarlet upon agar, reddish upon potato.

" 7. " pink " " scarlet " "

" 8. " pink " " scarlet " "

* this is the typical Bacillus indicus color.

The above table shows not only the variations upon potato and agar respectively, but the tendency toward the formation of darker pigment upon potato. This is not infrequent with bacterial pigments.

In general one can say that the fundamental color of the growth is the same in any case; a red. The particular tint, is variable in the extreme. This is of importance in the classification of bacteria; while the general tendency upon the part of the chromogenic bacteria is toward variation in tint, yet the fundamental, predominating color is reasonably constant.

The form of growth upon potato was practically the same in all the cultures; filiform or slightly spreading.

The elevation of growth was less marked than upon the agar slant, and ranged from a flat to a slightly convex growth. As before, these variations in elevation represent in general slight variations in the luxuriance of the growth.

The other characteristics, lustre, discoloration of potato and absence of gas production were comparatively constant.

Agar and gelatin colonies. It was more difficult to find characteristic colonies with the bacillus under consideration than was the case with the typhoid and colon colonies; the colonies of the latter bacteria were quite regular in outline, and agreed very well in almost every particular.

The colonies of Bacillus prodigiosus were quite varied in form, size and pigmentation. A greater diversity in form and color occurred than with any of the previously described organisms. There were, however, certain features that were reasonably constant and characteristic, and it was to these that one would naturally look for a description of colonies which would approximate the average colony appearance.

The cultures of Bacillus prodigiosus differed from one another in the extent of the location of the edges. The elevation and optical characters were constant. The general form of the colonies was that of a more or..

less pronounced rosette, although one could find all gradations between the round colony with almost entire edge to the deeply lobed, rosette-shaped colony.
The simpler, slightly lobed colony seems to represent most nearly the form that one would commonly meet with, and in general one can say that of all the colonies formed by a given species, the most symmetrical and least complicated are apt to be the usual form.

The colonies, then, are round or slightly lobed, the elevation usually slightly convex (this also holds true for gelatin colonies before they begin to liquefy) internal structure either slightly granular, or more often hyaline and amorphous.

The pigmentation of the colonies is less marked than upon the agar slantsprobably because of the fact that the layer of organisms is less thick.
The gelatin colonies were darker than the agar slant pigment, due to the fact that they were grown at 18° instead of 34°. The pigment is more pronounced at a lower temperature.

Crystal formation; culture number 1 formed rather large crystals upon agar, although they were not found

in any other media or formed by any other culture. In later experiments with the same culture they failed to form again.

Milk- every culture of *Bacillus prodigiosus* decolorized the litmus, usually almost completely. The organisms have the power to reduce nitrate solutions vigorously, and it is quite possible that this may account for the reduction of the litmus. On the other hand the organisms produce considerable acid in other media, and one may perfectly well ascribe this decolorization to an excessive acid production.

One great disadvantage of using litmus milk is the fact that the litmus is so readily decolorized; one must keep adding litmus if one wishes to follow the changes in reaction from day to day. Again, the litmus is not without a certain antiseptic action upon certain bacteria, inhibiting to a greater or lesser extent the luxuriance of growth. The advantages of litmus milk, however, more than offset the disadvantages, particularly in certain groups of bacteria in which the reaction changes from acid to alkaline in a characteristic manner.

In this investigation, all the results were checked

against plain milk; in the latter medium the reaction was determined by direct titration.

In addition to decolorizing the litmus, the bacteria coagulated the milk with considerable rapidity. The liquefaction of the coagulum was not constant; some cultures liquefied the casein to a considerable degree, while other cultures did not seem to change it at all.

The liquefaction of casein is not a very constant phenomenon with many bacteria, and does not in any sense indicate a difference in species merely because the individual cultures vary in this respect. The past history in many instances will influence this characteristic to a considerable degree.

Fermentation; none of the cultures produced gas in either dextrose, lactose or saccharose. The bacteria were capable of splitting dextrose, however, as is shown by the fact that every culture produced considerable amounts of acid in this medium.

The growth in the closed arm was very variable; some cultures produced a decided turbidity in the closed branch of the fermentation tube, some did not grow at all apparently, and some were doubtful. One could hardly say, even

after the lapse of week whether there was growth present in the closed arm or not.

This calls attention once more to the unsatisfactory character of growth in the closed arm in certain cases. Some bacteria produce a luxuriant growth, some grow moderately, some either very slightly or not at all; this may occur with different strains of the same species of organism. One is not justified in discarding entirely any bacterial characteristic because it is not constant in a given case, but one must choose for purposes of classification and identification of bacteria those criteria which are the most constant, and degrade the others to characteristics which may be of confirmatory value, after a given organism has been placed in its proper group. The theoretical considerations involved in the growth in the closed arm have been discussed in Part 1; the example noted above is a very good one illustrative of these conceptions, and is introduced for this purpose.

Nitrate reduction; all the cultures of _Bacillus prodigiosus_ reduced nitrate to nitrite and to free ammonia. In certain cases the reduction was so great as to cause a precipitate; a dilution of 1/[illegible] with nitrate free water

was used in such instances.

Indol production; considerable difficulty was experienced in obtaining solutions for the indol test which were free from the *prodigiosus* color; the pigment produced by this bacillus is not unlike the color of the nitroso-indol sulphuric acid compound, and one would be puzzled to distinguish between the two. Finally the expedient of growing the cultures at 40° was tried, and it was found that in such cultures no color appeared. All except culture number 3 produced indol.

Summary of *Bacillus prodigiosus* cultures.

The study of the prodigiosus cultures has helped to elucidate several points of paramount interest in systematic and descriptive bacteriology.

The most striking feature about a culture of *Bacillus prodigiosus* is its brilliant pigment.

The most noticeable feature about the cultures of this organism studied in this work was the multiplicity of shades of red when they were compared side by side, upon the same media.

This is not without interest in systematic bacteriology: many classifications depend among other character-

istics upon chromogenesis for division into groups.

The study of these cultures furnishes positive evidence for the interesting and important fact just mentioned; that while the *shade* or *tint* may vary, the *fundamental*, or *predominating* color is constant.

This seemed to hold good upon both agar and potato plants: upon blood serum pigment was not produced, except in a very few cases.

Cultural and Biochemical Characteristics; the liquefaction of gelatin, fermentation, nitrate reduction and indol production were very constant with every culture of *Bacillus prodigiosus*.

It seems very probably from this study that *Bacillus prodigiosus* and *Bacillus indicus* are not two species, as was originally supposed, but at best only two sub-varieties of the same organism; their only difference, as noted in a comparative study of the two organisms is a tendency to form different colored pigments: even this is not constant, and they are constantly changing from one to the other color, even in the same culture.

Bacillus Pyocyaneus.

Bacillus pyocyaneus (Pseudomonas pyocyanea) is an organism of extreme interest to the bacteriologist from every point of view.

Not only is it pathogenic for man and the lower animals, but it is also of great significance from the standpoint of classification, representing as it does almost the extreme of Cultural and Biochemical possibilities.

Bacillus pyocyaneus liquefies gelatin, blood serum and coagulates and liquefies casein. The enzyme which causes the latter change is rather different from the rennin produced in the human stomach; the former acts best in alkaline solution, and one finds the terminal reaction of milk usually alkaline.

The chromogenic and fluorescent properties of this organism are marked.

The earlier observers believed there were two pigments produced;

1- pyocyanin: soluble in chloroform and ether, blue-green in color, insoluble in water.

- a fluorescent, greenish pigment, soluble in water, but not soluble in ether and chloroform.

3- a red-brown pigment.

The latest researches seem to indicate that there are but two pigments, the pyocyanin, characteristic of Bacillus pyocyaneus, and the fluorescent pigment. The red-brown pigment is but a modification of the pyocyanin. This modified pigment is often spoken of as Pyoxanthose.

The pyocyanin and its associated modified pigments produce a range of color which has been the cause of much confusion among bacteriologists: many have fallen into the error (a perfectly natural one) of interpreting these variously colored chromogenic products as of specific importance. Various 'varieties' of Bacillus pyocyaneus have been described according as one or another of these pigments have seemed to predominate.

Wassermann has published in Kolle und Wassermann's Handbuch der Pathogenen Mikroorganismen, vol.3,p.47 , an excellent resume of the present condition of our knowledge relative to this point: the results of the work upon this organism confirm his results perfectly, and I can ot do better than to quote his results in this connection.

"Auf Agar-Agar ausgestrichen wächst der Pyocyaneus besonders bei Bruttemperatur sehr üppig in Form eines feu-

ichteten, ziemlich dicken, grauen Rasens, der durch Farbstoffproduktion den gesamten Agar grün färbt und aufhellt. Nach 1-2 Tagen verschwindet gewöhnlich diese grüne Farbe und weicht einer rotbraunen (s.u.) Die Farbennuance des gebildeten Pigmentes kann sehr schwanken, hellgrün bis blau oder grünlichbraun. Sie hängt zum Teil von dem betreffenden Stamme, zum Teil von dem Nährboden ab. So sah ich öfters, dass ein und derselb Stamm auf einem Agar grüne, auf einem frischen gleichartigen Agar dagegen blaue, auf einem dritten grünlichbraune Verfärbung erzeugte. Offenbar genügen sehr geringe Veränderungen des Nährbodens hierzu, und es ist nicht berechtigt, auf Grund solcher geringer Unterschiede in der Farbstoffproduktion verschiedene Rassen des _Bacillus pyocyaneus_ aufzustellen (s.u.)"

More will be said in Part 3 concerning the changes in color of the pigment as one makes observations from day to day.

Cultural and Biochemical Characteristics.

Aside from the variations noted in Chromogenesis and Fluorescence, the agar slant cultures of _Bacillus pyocyaneus_ showed comparatively little difference upon the whole.

With but two exceptions cultures number 4 and 5, the growth was filiform, with slight tendency to spread. The two cultures mentioned were echinate, although this form of growth is of the same order as the filiform growth, and has no particular significance.

The cultures were flat, surface smooth, although at times there seemed to be a tendency toward an umbilicate elevation. This was marked in culture number 4. At times, too, the surface was slightly wrinkled, or even punctate. As a rule the growths were shining, although certain cultures seemed to have an iridescent luster. This was very marked with certain media to be described later , in Part 3.

The cultures were as a rule devoid of viscosity, and opaque.

Chromogenesis and Fluorescence have been described: two cultures however, deserve special mention. Cultures number 5 and 5 did not produce either pigment or fluorescence. Their growth was very similar to the characteristic growth of <u>bacillus coli</u>, except for the fact that they had a more or less pronounced iridescent lustre.

Gelatin stab cultures: _Bacillus pyocyaneus_ liquefies gelatin with great rapidity, especially when the cultures are in a state of rapid vegetative reproduction. The findings with the 17 cultures examined in this work were no exception to the rule. Each and every culture agreed perfectly in this respect, although the form of liquefaction showed every possible variation.

A few cultures produced a pellicle upon the liquefied gelatin.

A few organisms showed a well marked fluorescence upon gelatin. It is particularly interesting and significant to note that of these cultures, one, number 6, did not exhibit and fluorescence upon the agar slant even after the tenth day. There is no explanation for this remarkable variation.

Culture 6 grew well both upon gelatin and agar; it liquefied the gelatin, and produced its characteristic fluorescence upon that medium, while upon agar there was not the slightest trace of fluorescence. This becomes the more extraordinary when one remembers that the organism had in common with the typical cultures the same preliminary cultivation, and was inoculated upon samples of

media of the same lots in each case.

Broth: A few cultures produced a pellicle upon this medium. It is interesting to note that there is no parallelism between the formation of a pellicle upon broth and upon the liquefied gelatin.

The cultures of <u>Bacillus pyocyaneus</u> furnish the first instance in which the organisms were deficient in some characteristic: the majority of the descriptions of this organism agree that the formation of a pellicle is the rule rather than the exception.

The turbidity and sediment were not characteristic. Cultures number 2 and 12 produced fluorescence, while the remainder were devoid of this characteristic.

Blood serum; blood serum was liquefied by every organism representing <u>Bacillus pyocyaneus</u>; the serum was usually stained greenish-yellow, due to the formation of pyocyanin. This pigment penetrated to a considerable degree the surrounding unliquefied serum.

Potato; the form of growth was quite constant upon potato; the general type was filiform, non-characteristic growth. The extent of the growth varied from a limited,

narrow stripe to a rather broad band. The elevation was also varied; ranging from a flat thin layer to a raised, decidedly capitate surface. The surface was a shining, smooth production as a rule.

The potato showed three types of discoloration;

1- a single darkening.

2- a more or less diffuse blue-green discoloration,

3- a few cultures showed a tendency to form a pinkish discoloration of the medium adjacent to the culture in addition to the blue-green discoloration previously mentioned.

Chromogenesis; the usual color differed somewhat from that produced upon the agar slant. Although bluish and greenish pigments were seen in a few instances, the usual appearance was a rather brownish surface. Some of the organisms produced absolutely no pigment upon potato, yet these cultures were among the most active in the general reactions, and grew luxuriantly upon this medium.

The so-called chameleon phenomenon' was not at all constant, although regarded as of diagnostic importance by many observers.

Gelatin and agar colonies; the general characters of

the gelatin colonies were observed before liquefaction begun. After the medium began to soften, the colonies assumed a saucer-shaped appearance, with the central part opaque, due to an accumulation of bacteria. It should be stated that the form of liquefying colonies upon gelatin plates are non-characteristic, and for this reason, do not appear either in the descriptions, or upon the tables themselves. The best that can be done in such a case is to make the description before liquefaction has begun. The agar colonies were very constant in every particular-form, elevation, character of the edge and the microscopic structure.

Milk; The cultures of Bacillus pyocyaneus formed a soft coagulum which rapidly liquefied, leaving a turbid, brownish limpid liquid, which was alkaline as a rule in reaction.

Fermentation; the cultures did not produce gas in dextrose, lactose or saccharose. They agreed in producing no acid fermentation of the dextrose.

Nitrate reduction; nitrate was reduced by these cultures both to nitrite and to free ammonia. The reduction was very great in some cases.

Indol production; the majority of descriptions do not attribute to *Bacillus pyocyaneus* the power of forming indol. Here again, the preliminary cultivation, and also the excellence of the sample of peptone seem to show that certain strains, at least of this bacillus may, and do form indol, if the conditions are favorable.

The typhoid cultures, it will be remembered, with one exception formed indol; the cultures of *Bacillus pyocyaneus* were about evenly divided, half formed indol in considerable amounts, the rest did not give even a trace of this substance.

With the study of this organism, the first part of the cultural work undertaken in this research is completed. After summarizing the results, the subject of variations occurring when the same organism is placed upon different media of the same kind will be discussed.

Summary of Part 2.

Investigation of different cultures of the <u>same</u> bacterial species from different sources upon the <u>same</u> lots of media under the same conditions.

The object of this portion of the investigation was to determine by direct experiment if bacteriologists, in spite of differences in method and technique, agree in essential points upon those characteristics which are necessary to define a bacterial species.

Cultures of four common, well known organisms, *Bacillus coli, typhosus, prodigiosus* and *pyocyaneus*, were obtained from a number of laboratories, and in addition a few cultures from recent autopsies.

The various cultures were plated out, to insure purity, given a careful preliminary cultivation and inoculated into a series of media, made according to the American Public Health Association methods. A few slight modifications were made in the methods of making media, which have been referred to in the text above.

The cultures were examined upon the first, third, seventh and tenth days; only the tenth day records are considered in this connection.

The results show several interesting and important facts;

1- With one exception, the cultures agreed in essential characteristics with the type species of which they were representatives.

2- The cultural reactions of the bacteria, while not absolutely identical, were of such a nature that one could rely upon them within certain limits for descriptive purposes; they were characteristic, although not specific, for each species of organism.

3- The cultural variations were slight, usually, and were apparently due to slight variations in the relative luxuriance or intensity of growth; never to differences in method of reproduction, or mechanics of colony formation.

4- The biochemical reactions divide themselves quite sharply into two groups; to the first group belong those reactions which were constant for each culture examined, and would probably be constant for any species of bacterium. To this group belong the production of gas or of acid in fermentation solutions, and probably the nitrite reduction.

To the second group belong those reactions, which while

not absolutely constant for all bacteria, still are of great importance in certain groups; such reactions are not of general classificatory value, but of specific importance. They are essential for the identification of certain groups or certain specific bacteria. To this class belong the reactions in milk, the free ammonia production, indol production and growth in the closed arm.

Based upon the evidence herewith presented, then, one may distinguish two classes of reactions; those which are of diagnostic importance, and those of confirmatory importance. The former constant for all bacteria, and suitable for use as criteria for division of bacteria into groups: the latter of diagnostic importance in certain groups or species. The latter class will not be of material use for general divisions of bacteria, but are limited in their application.

Part 111.

Investigation of the *same* species of bacterium upon *different* media under the same conditions to study;

1- influence of reaction of the medium.

2- influence of the concentration of the medium.

3- influence of temperature and moisture.

4- time factor in final result.

5- influence of certain albuminous substances.

6- the 'personal factor' in determinations of the *same* cultural phenomena by *different* observers.

7- value of various cultural and biochemical characteristics for bacterial classification, based upon their relative constancy.

In the previous chapter of this dissertation the methods employed in species determination have been discussed; their relative importance and comparative accuracy ascertained, and finally the variations occurring with different strains of the *same* bacterial species upon the *same* cultural media elucidated.

It was shown that even when the *same* media were used as nutrient substrata the various organisms showed variations in cultural reactions which, while they were of

the same order, yet were noticeable; the explanation is probably to be found in the well known principle of 'variation'- one of the most striking and characteristic attributes of living things.

It remains to determine several other variables; of which the most important are those tabulated at the beginning of this chapter; finally, to apply the results of these investigations to the ultimate object of this work, the classification of bacteria.

The present portion of the work has to do exclusively with cultural phenomena; morphological considerations are to be considered later.

The first cultural characteristic to be considered will be colony formation. It may excite surprise to find that this will be considered briefly, since it is upon this characteristic that one relies for separation of bacteria from one another.

Mention of the principle features of colony formation have already been made- the characteristics to be observed are form, elevation, edge, topography, optical characters (or the appearance of the colony when viewed by transmitted and reflected light) and chromogenesis.

for bacterial nourishment, even though they may be quite as rich in nutriment, than are fluid media. This is almost a self evident fact, and needs no further consideration. Yet one must utilize such solid media for the demonstration of colony formation.

The mechanics of colony formation have been investigated by certain observers, but as yet the phenomena are not satisfactorily explained. One method of research has been to section mature colonies, and study the arrangement of the cells; this has not been productive of valuable results, and is inferior to the 'Hanging Block' method of Hill, which will be referred to again. Here one may actually see the organisms proliferating, and assuming their characteristic arrangements in situ. The aggregates, while apparently at first glance, are without definite arrangement, can be shown to be the resultant of an orderly, almost definite sequence of growth.

Chester, in a communication to the Society of American Bacteriologists, (Philadelphia, Dec. 1903,) in a paper entitled " Notes on the _Bacillus subtilis_ group," describes the formation of filamentous and flocculent colonies, or at least the initial stages; such colonies are characterized by the fact that they have a definite arrangement of the

cells in long chains; these chains are arranged in such a manner that they give a characteristic appearance to the colony.

There is then a certain, not unimportant relation between the method of reproduction of the bacteria, and the form and structure of the colonies they produce.

Usually the environmental conditions are not parallel, the varying external conditions having a corresponding influence upon the form of the colony; a specific example will illustrate the general principles involved.

Every bacteriologist is familiar with the variations occurring in colonies of Bacillus coli. Macroscopically, the organism may produce a flat, spreading, more or less elevated growth, usually with the centre distinctly raised.

Microscopically, the whole colony may be uniformly granular, or the centre may be composed of large granules with a finely granular periphery; in certain cases the centre may be quite opaque, while the remainder is translucent, or even transparent at the edges.

When one considers the relation of the original bacterial cell or cells to the medium, the explanation will be

apparent. Consider that a cell happens to lodge beneath the surface of the medium; the resistance of the medium is practically the same in all directions, and the organism will have to overcome resistance in whatever direction it happens to grow. The first daughter cell will in all probability lie in the plane which is a prolongation of the long axis of the mother cell. This may be parallel to the surface of the medium, and theoretically division might go on indefinitely at the level of the mother cell; it is probable that various conditions tend to change the direction of growth, until finally the cells reach the surface provided the original cell was not too deeply imbedded at the start. Oxygen in aerobic forms is not without chaemiotactic influence; the nearer the surface the organisms reach, the more plentiful the supply of this gas. This slight factor is enough to influence the direction of growth, and finally the organisms will reach the surface. During this time waste products are accumulating, and if the original colony were much below the surface, by the time the daughter cells reach the top, they are surrounded by the products of their excretion, and indeed as a rule, deeply submerged colonies are rarely

spreading when they reach the surface; further growth is limited.

If on the other hand the bacillus lie originally upon the surface, a flat, spreading growth usually results, because the resistance in the horizontal plane is minimal, and no time is lost pushing aside or digesting the super natant material. The greater lateral extent of growth is due then, probably, to the fact that the organism extends without resistance, and the waste products are spread over a large area rather than being concentrated in a relatively small space.

This, then, is a reasonable explanation of the difference which one notices in the same plate, with a pure culture of the same organism; thin, flat colonies produced by organisms lying upon the surface; elevated, limited colonies when the cell lies beneath the surface. Of course since the internal structure depends upon the thickness of the layer of bacteria, this characteristic, too, will vary as one or the other condition obtains.

The limits of variation of the same culture upon a plate are considerable, and the writer has found repeatedly that with ordinary media, the limits of variation from one to another [illegible] are no greater than those [illegible] the

same medium as one records the characteristics of the various colonies. The relative luxuriance of growth may vary, but the form, and general characteristics are relatively the same in any case where the medium is suitable for bacterial growth.

For this reason, the descriptions of colonies are subordinated to a careful study of the characteristics of slant and stab cultures, where one has no average colony to choose for descriptive purposes. It is necessary to determine the relative value of observations made upon slant and stab cultures that they may on the one hand receive a proper amount of attention, and on the other hand that their actual value as diagnostic characteristics be not exaggerated.

Investigation of the characters of certain bacteria upon slant agar and in the gelatin stab.

The organisms used in the previous portions of this investigation, Bacillus coli, typhosus, pyocyaneus and prodigiosus, were used in this portion of the work.

They were given preliminary cultivation as before, and inoculated with the usual precautions into various samples of gelatin and agar.

These media were varied in reaction, and to a certain extent in composition. The reactions and composition may be found in Part V. Only those media which were composed of ingredients other than gelatin or agar, meat extract and peptone will be mentioned here.

Agar 1; meat-extract agar as above, with 1% glycerine.

Agar 4; meat juice instead of meat extract.

Agar 7; peptone and agar merely.

Agar 8; glycerine, peptone and agar.

Agar 9; starch and agar.

Agar 10; agar and blood serum.

Gelatin 3 and 4; meat juice instead of meat extract.

Gelatin 3 contained 8 % gelatin, gelatin 4, 15 % gelatin.

One of the principle objects of this portion of the work was to determine the possible variations in making media from the <u>same</u> ingredients by different observers. Four individuals, accustomed to making bacterial media, made both agar and gelatin at the same time, from the same lot of material.; if such variations occur, as have been noted above, one would expect similar slight variations to obtain in all bacterial work.

Whipple (loc. cit) has studied the variations occur-

ring in relations made from different samples of this material made by the same observer, and also he studied the variations occurring when different men made gelatin, and the same observer inoculated the various samples with transfers from the same culture. He finds great variations in groth form; variations great enough to furnish ground for the elimination of gelatin stab cultures from descriptive bacteriology.

It will be much simpler to discuss the general reactions of each species upon each medium first, then consider the various subjects listed at the beginning of this section separately. By so doing, one can get a better idea of the growths as a whole, then one can better interpret the variations that may occur in the light of their probable relations to the composition of the media, etc.

Bacillus coli. (Tables 5-11)

The variations occurring with the first 10 cultures of this organism, described in Part 11 were studied upon six different agar and five gelatin samples.

Results of the 10 th day observations. See Tables 6, 7. Culture 1; growth filiform, flat, grayish, with shining lustre; viscosity upon all media except Agar 4; (it will

be remembered that this agar was made from meat juice, according to the procedure recommended by the American Public Health Association). Upon this medium the culture grew more luxuriantly, and the color of the growth was a pure porcelain white instead of the gray upon other agar samples.

Cultures 2 and 3 were precisely the same as No. 1. in essential characters.

Culture 4. showed a tendency to spread; this was particularly marked in agars 3 and 4.

Culture 5 also showed the same spreading growth.

Culture 6. The spreading was even more marked with this organism; otherwise it was similar to the preceding.

Culture 7 grew luxuriantly upon all the agars except Nos. 1 and 4. This is the first instance so far in which the meat juice agar has failed to furnish the most favorable medium, as is shown by the relative luxuriance of the streaks. The other medium failing to give a spreading growth was No. 1 which contained glycerine in addition to the regular ingredients.

Culture 8 grew luxuriantly upon all the agars except the one containing glycerine.

cultures [illegible] and 10 grew less luxuriantly, but still showed some tendency to spread.

By comparing these growths the tendency toward a spreading, white growth upon agar 4 and the limited growth upon agar No. 1 are the only noteworthy deviations from the average appearance. Lustre, elevation, optical characters topography and viscosity were practically identical upon all the media.

Colon cultures in the gelatin stab.

The results of these growths can be summarized very briefly; culture 9 liquefied gelatin, as has been noted in Part 11. The remaining nine cultures did not liquefy the gelatin, produced as a rule a flat surface growth, and practically every known form of line growth.

Summary of colon cultures.

Influence of reaction of the medium; within the limits of reaction studied, -0.3 to + 1.2 no definite differences could be detected in any of the growth forms of the colon bacillus. The organisms to be sure varied to a considerable degree, but the variations produced were not greater than those obtained with the <u>same</u> cultures upon the <u>same</u> medium. Nothing definite is to be learned from the reaction

of the media with these cultures it is probable that the variations that did occur are to be attributed in part at least to the individual idiosyncracies of the cultures.

Influence of the concentration of the medium. Nothing conclusive is shown beyond the fact that other things being equal, those media which are considerably less concentrated with respect to their solidifying constituents, gelatin or agar, were the ones upon which the growths were the more spreading.

In the case of gelatin, the character of the line growth varies almost directly with the concentration of the medium. The less solid the gelatin, the more spreading the line growth. This factor (solidity) is not quite the same as the melting point in its effect, as Whipple has pointed out (loc. cit) . The latter is variable; the melting point may be extended over a degree or two in some instances, or the mass may liquefy almost sharply at a particular temperature. The solidity, on the other hand, is a definite quantity.

Influence of temperature and moisture; the relation between temperature and growth of Bacillus coli is a very simple one: up to 40° the rapidity of growth varies almost

directly with the temperature. The higher the temperature up to the limit stated, the more vigorous the growth.

Influence of moisture; this subject may be considered under two heads; moisture in the cultural medium and in the environment outside the cultural media.

The moisture upon the surface of the media upon which cultures are growing is very apparent, whether the moisture is visible to the eye or not, by the extreme tendency upon the part of the colonies to become spreading. In many instances one sees examples of this upon the agar slants- as the result of careless handling the condensation water flows up the sides of the tube. In a few hours there is a delicate film of organisms spread all over this moistened area. The same applies to freshly slanted surfaces before the excess of moisture has flowed off, or evaporated. Of course cultural descriptions of such slants are quite worthless for descriptive purposes.

Moisture in the environment. This factor is too often neglected in bacteriological work, although it is of the greatest importance.

The writer has found repeatedly that re[illegible] take place with much more certitude, particularly if they be

of such a nature that a week or more is required for their development- if cultures be kept in incubators in which the atmosphere is constantly saturated with aqueous vapor.

In a series of parallel experiments cultures grown under conditions the same except that in the one case the incubator was supplied with moisture, in the other no moisture was present, the organisms were in much better condition in the former than in the latter case. The reactions were more complete, and the organisms grew much better after two weeks in the moist atmosphere than in the dry.

Plate cultures may be kept two or three weeks and still be in comparatively fresh condition in moist incubators. This applies equally well to slant and stab cultures.

Time factor. Fuller and Johnson (loc. cit) found that two weeks, the usual time for bacterial incubation, was practically no better than ten days so far as completeness of reactions was concerned, for bacterial descriptions; during this time bacteria will produce almost all of their characteristic reactions upon almost all media, provided the organisms be growing luxuriantly at the start.

The time one allows bacteria to develop before making final observations depends upon two factors; a- the time

in which the greatest possible number of reactions shall be complete by the greatest possible number of bacterial species, and, b- the time of final observation shall be soon enough after inoculation so that one shall not be handicapped by the accumulation of culture in routine work. The chances of dessication, contamination and regression of characteristics should be taken into consideration.

Ten days, the time allowed by the above mentioned authors, seems to be the maximum interval which one can logically allow for observations, and this period has been found to suffice in this work for bacteria to bring about their characteristic changes.

If one gives organisms careful preliminary cultivation, and inoculates them into media under optimum conditions as far as possible, in almost every instance, no changes of importance will occur after the tenth day. In fact, all the organisms studied in this investigation were complete after the seventh day. (see tables illustrating the changes occurring from the third to the tenth day inclusive).

Influence of certain albuminous substances; Dr. Wesbrook, in a private communication, suggested the possibility of using blood (or blood serum) as a possible substi-

tute for meat extract or meat juice in the preparation of nutrient gelatin and agar or even bouillon.

If good serum be available, the chances of duplicating the proteid constituents of media are greater than can be the case when meat juice and possibly meat extract is used. The salt content in meat extract is the chief variable, and of course this would be eliminated.

Acting upon this suggestion, I have made samples of agar using blood serum as the proteid basis. Agar 8 consists of 1 % agar, 10 % serum, reaction + 0.1 and agar 10, 1 % agar, 10 % serum, 1 % peptone. Ox serum was used in preference to horse serum, chiefly because it was more available, although many writers claim the latter contains more 'muscle sugar' (glucose?)

This is a step towards synthetic media, and the results of growth upon these agars are correspondingly interesting. By referring to tables 6 to 22 inclusive, it will be seen that <u>Bacillus coli</u> grew quite as luxuriantly, and with the same general characteristics upon agar 8 as upon agar made from either meat extract or meat juice. One may, however, note that the growth took place rather more quickly upon the meat juice agar; the latter medium was the best of all as far as general results were concerned.

The serum agar was quite as good as the meat extract for all purposes, and is worthy of further study. Certain peculiarities shown by growth of chromogenic organisms will be discussed later.

<u>Bacillus typhosus</u>. (Tables 12-19)

<u>Agar slants</u>.

Culture 1; grew luxuriantly upon all the agars except agar No. 1, which contained, it will be remembered, glycerine in addition to meat extract and peptone.
Cultures 2,3, grew less luxuriantly than culture 1.
Cultures 4,5,and 6 grew very similarly to culture 1.
Cultures 7,8,9,10 and 11 grew very luxuriantly indeed.
Here again, it is interesting to note that the agar containing glycerine was the one upon which the poorest growth appeared.

Gelatin stabs.

None of the cultures liquefied gelatin; they produced as a rule a non-characteristic, flat surface growth, and as usual showed every type of line growth.

The typhoid growths resembled each other even more than did the colon cultures. The various differences in composition affected <u>Bacillus typhosus</u> in precisely the

same way as they did the colon cultures.

The glycerine seemed with the ones studied at least, to decrease the relative luxuriance of growth, while the malt juice furnished nutriment upon which the organisms grew more luxuriantly than upon the other media. The serum agar was at least as good as the malt extract in all the determinations.

In general, then, one may say that the above studies show that reaction is practically without effect, as long as the limits are within the ordinary range to which bacteria can derive nutriment, and grow; the malt juice is rather better than meat extract, and blood serum is intermediate between the two. As will be seen later, those organisms producing pigment are the ones which are chiefly affected.

These statements must not be interpreted to mean that the generalizations above are absolutely true for all bacteria, but since these organisms are ones which occupy an average position in bacterial possibilities, one can conclude that at least the majority of forms are certain to follow these cultures in their general behaviour.

Organisms like the cholera vibrio will not grow upon media which are acid, and the gonococcus will not grow

upon ordinary media at all, but the latter are extreme cases and do not enter into this consideration.

Summary of Cultures of _Bacillus typhosus_.

Influence of reaction; within the limits of reaction studied in this investigation, (-.0.2 to + 1.8) no differences attributable to this factor could be detected. The variations which did occur were in no instance consistent upon any one medium; consequently no explanation of the variations could be explained upon this basis.

Influence of the concentration of the medium; again no definite results were obtained- the growths were very similar upon media having the greatest range of reaction; both of gelatin and agar.

Influence of temperature and moisture; the same factors entered into the growth of _Bacillus typhosus_ as was the case with _Bacillus coli_- quicker and more luxuriant at 37° than at 18-20°. The organisms were more typical in their reactions and retained their vitality better in moist than in dry atmospheres.

Time factor; the growths attained their full cultural possibilities in less than 7 days-observations upon the tenth day growths were hardly distinguishable from those of a week.

influence of sera in albuminous constituents; one could hardly distinguish the growths of _Bacillus typhosus_ upon serum agar from corresponding colonies upon the regular meat juice or meat extract agar. This is precisely what was noted with cultures of _Bacillus coli_; yet the medium (serum agar) is very simple in composition, being composed of 1.5 % agar and 10 % ox serum, extremely simple in composition when compared with the other media employed. With the non-chromogenic organisms studied, the various agencies indicated at the beginning of the chapter are without great effect. Particular attention is called to the fact that the influence of reaction is without the slightest consequence upon the growth of the organisms studied so far; the literature of bacteriology is full of researches upon this subject, and while undoubtedly the reaction has considerable influence upon the numbers of bacteria in a given source, and while again, the ultra acid or ultra alkaline media will seriously interfere with the numbers of bacteria growing upon a particular medium, yet the results seem to indicate that while the reaction was varied from -0.2 to + 1.2 normal acid to the litre, the resulting growths were remarkably constant,

and that one could employ relations within these limits, and be reasonably sure of obtaining concordant results.

Bacillus prodigiosus.

(Tables 20-24.)

It will be more convenient to tabulate, the general findings of the chromogenic organisms, so that one may more readily see the variations which occur as the conditions are varied.

Agar 1; form of growth filiform, elevation convex or raised, lustre shining, chromogenesis usually red; viscosity varied, but more marked than upon the preceding agar; optical characters opaque; topography smooth.

The color was red, but the red usually ascribed to *Bacillus indicus*; a red-brown or sealing wax instead of the scarlet characteristic of *Bacillus prodigiosus*. The condensation water, however, was colored scarlet, like the typical prodigiosus dark red.

Agar 3 was essentially like agar 2.

Agar 4: form of growth spreading; elevation flat or raised; lustre shining; optical characters opaque; viscosity varied; chromogenesis; as a rule the pale red or pink occurred, with a tendency toward the formation of scarlet in the condensation water.

Agar 7; form filiform or echinate, and rather limited; elevation convex to hemispherical; lustre shining, or metallic; optical characters opaque; color sealing-wax to scarlet; the former predominated. The organisms grew less luxuriantly upon this medium than upon the preceeding.

Agar 8; form filiform; elevation raised or convex; lustre shining metallic or dull; optical characters opaque; voscosity negative; the color was almost invariable scarlet.

Summary of agar cultures; the form of growth varied from a rather limited, filiform to a very luxuriant echinate streak. The most luxuriant growth occurred upon the meat juice agar, agar 4.

The elevation was usually decidedly convex indicating a luxuriant growth.

The lustre was usually shining, although a decidedly metallic appearance, simulating closely the peculiar sheen of fuchsine appeared in some cultures. Upon agar 8 the lustre was rather dull.

All the cultures were opaque; no light could be transmitted through them.

the viscosity was extremely varied; this was partly due to peculiarities of the individual cultures and partly to the characters of the media upon which the organisms were grown. Agars 2 and 3 were the ones upon which the viscosity was most marked.

Chromogenesis; there were three distinct types of color; a pink, which was the predominating type upon agar containing meat juice; a scarlet, prodigiosus color, which occurred in media containing glycerine, and finally the brown-red or 'sealing-wax' color said to be characteristic of Bacillus indicus. The latter occurred in those agars which contained meat extract particularly. Together with the pigment upon the surface there was a tendency to form a scarlet color upon the surface of the condensation water.

To summarize; not only is there a rather definite color reaction upon the different media, varying as the protein of meat juice, extract serum, or containing glycerine, but also the organisms themselves have peculiarities which either accentuate or take away from the color impulse furnished by the medium upon which the cultures are grown. Agars 5, 7 and 8 produced deeper scarlet than the other media.

gelatin cultures.

It will be necessary not only to tabulate the general results of growth in the gelatin stabs, but to record the relative frequence with which each characteristic occurs upon any one medium; this is necessary because, unlike the agar growths, there is no general type to which one may refer the various growth-forms.

The chromogenic reactions were marked, and will be included even though they do not appear as a rule in descriptions of the gelatin stab.

Gelatin 1; form of liquefaction; infundibuliform,6; napiform, 1; surface growth; (pellicle) 2, ring, 1. color. scarlet, 4; pink, 2; reddish,1. the tendency toward a scarlet color was very pronounced.

Gelatin 2; form of liquefaction. infundibuliform,5; stratiform, 2. pellicle; 3,ring,2. color; pink,2; scarlet,1; brown-red, 3; reddish,1.

Gelatin 3; form of liquefaction: infundibuliform,4; stratiform, 3. pellicle: 2,ring formation,2. color: pink,2; scarlet,1; brown-red, 3; no color,1.

Gelatin 5; form of liquefaction: infundibuliform,4; stratiform,3. pellicle: 2; ring,1. color: brown-red, 3; scarlet,

5; pink, 2.

Gelatin 6: form of liquefaction; infundibuliform, 4: stratiform, 3. Pellicle: negative, no ring. Color: scarlet, 3; brown-red, 1; pink, 1; no color, 1.

Summary of color production.

scarlet, 11; brown-red, 11; pink, 5; reddish, 1; no color, 3.

Form of liquefaction;

infundibuliform, 23; stratiform, 11; napiform, 1.

Summary of <u>Bacillus prodigiosus</u>.

Influence of reaction; mention has already been made of the fact that the color varied greatly in the different cultures, even upon the <u>same</u> medium. It will be necessary to try and trace the origin of these variations from the tables. (Tables 33-36, also table 24).

Agar 1; all the cultures grew luxuriantly as filiform, decidedly convex, shining, smooth opaque growths. The general morphology of the slant growth was non-characteristic. Nearly all the cultures, however, were dark red or scarlet in color. This was true of 75 % of the organisms examined. The remainder were lighter in color, but still of a decided red tint.

Growth upon agar 3 was almost identical. Both of these media contain glycerine.

The reaction of these media was quite different. Agar 1 was slightly acid (+0.1) while agar 5 was quite acid, (+ 0.5) apparently this amount of acidity was without effect.

Agar 2 and 3; Growths upon these media were brown-red, with a tendency toward the formation of scarlet condensation water. There were no differences in the character of the growths.

The condensation water reaction is peculiar, and seems to be almost a connecting link between the color characteristic of _Bacillus prodigiosus_ and _Bacillus indicus._ The generally accepted view concerning the condensation water of agar slants is that it has properties different from either the fluid or the solid constituents of the medium.from which it is derived. The color is extremely different from that either of the broth or the agar, and this would seem to bear out the generally accepted view.

Agar 4; this agar was acid, (+ 1.2) hence quite different inreaction from the agars described above. The pigment

was very variable upon the medium, ranging from the brown-red to scarlet. The predominating color, or at least the color which appeared most frequently, was pink. Both in the cultures showing scarlet, and brown-red, the condensation water had this pinkish tint. Upon the whole, the chromatic variations were greatest in this medium.

Agar 7; reaction + 0.5. The general color was similar to that produced upon agars 2 and 3,- brown-red with scarlet condensation water.

Summary of influence of reaction;

Practically no logical conclusions can be drawn from the results obtained. Reaction was without influence- to be sure the growth upon Agar 4, (reaction + 1.2) showed a tendency to vary in color, but the variations were within those produced by agar of neutral reaction. In general, from these experiments, one may say that the cases in hand showed no definite reaction in color production upon the part of the organisms to differences in reaction ranging from -0.2 to + 1.2.

Influence of temperature and moisture.

It was found that the influence of temperature was less marked with Bacillus prodigiosus than is usually the

case according to descriptions. Bacteriologists agree as a rule that the organism does not produce pigment at 37°.

The cultures studied in this investigation, after preliminary cultivation, produced pigment in every instance. It should be remarked that the pigment was less intense than corresponding colors obtained from parallel cultures grown at 20°, however.

Influence of moisture.

The presence of moisture was not without effect upon cultures grown in the incubator; it was found by experiment that the production of a scarlet color in the condensation water, referred to above, took place slowly, and in some instances not at all, at least until the tenth day, if the organisms were grown in the dry incubator. The cultures seemed to dry up and die before the characteristic color developed.

Time factor.

The ordinary gradual development of cultural characteristics, including the production of pigment occurred with <u>Bacillus prodigiosus</u> as one would expect. With the exception of the fact that the pigment was less intense, less 'saturated' at 37° than at 20°, the cultures grew faster

at this temperature than at 20°, but even at the lower temperature, the characteristic reactions were present at the end of the seventh day.

Influence of composition of the media.

The above factors have demonstrated that neither the reaction, concentration, temperature, moisture or time factor have practically nothing to do with the variations in the color production of Bacillus prodigiosus. A careful examination will show, however, that there is a relation more or less constant, between the albuminous (proteid) constituents and the chromogenesis of this organism. Agar 1 and agar 8, containing glycerine, peptone and agar in common, showed almost invariably a dark scarlet pigment. This is not due to agar, for agar is a common constituent of all the media; the color is not uniformly dark: nor is it due to the peptone, because Agar 7 contained only agar and peptone. As a rule this dark pigment was found only upon media containing glycerine. Hence one must assume that this substance has a direct effect upon the color produced by certain strains of Bacillus prodigiosus. Agar 2 and agar 3, containing meat extract, peptone and no glycerine, made a brown-red (indicus) pigment. This was

also the rule; in addition, these media were the ones upon which the viscous, mucin-like material appeared in most of the cultures. Since no other sample of agar used in this portion of the work contained meat extract, and the other ingredients were common to other agars, one must assume that the meat extract is the exciting cause for these phenomena. Again, the tendency to form a brown-red growth with the scarlet condensation water was most highly developed in these agars, although the condensation water in other samples was to a certain extent productive of this characteristic. One would hardly be justified in attributing the condensation water color to meat extract, but it is to referred to the general properties of this portion of the agar slant, since all agars were characterized by the scarlet color to a greater or lesser extent.

Agar 4; containing meat juice, peptone and agar, having a reaction of + 1.2, was characterized by the presence of a red or pink color. The tendency toward the formation of definite pigment, however, was less marked upon this medium than upon the preceding. One cannot make as definite statements with meat juice as with the other media, since the reaction is quite different, and the chromogen-

axis was variable.

Agar 7. containing peptone as the only proteid constituent, did not differ markedly from agars 2 and 5, except that the tendency toward the formation of mucinous substance was absent. This is a bit remarkable when one considers the extreme simplicity of the medium, when it is compared with the other samples of agar.

Agar 8. checked almost identically with agar 1, except the pigment was very slightly lighter, when the two were compared side by side. The medium contains glycerine in common with number 1, and in addition peptone and agar. This seems to be quite conclusive in demonstrating that glycerine is <u>the</u> factor in determining the darkness of the pigment of *Bacillus prodigiosus*, as far as the media studied in this connection are concerned.

Summary.

The various samples of agar, upon which various strains of *Bacillus prodigiosus* are grown, agree closely in the general morphology of the colonies upon their slanted surfaces. Not only the form, but the topography, elevation lustre and optical characters are very similar.

Slight, apparently constant, differences were noted,

particularly in agar 4, upon which the organism had a distinct tendency to spread; agars 2 and 3 produced variable amounts of mucinous substance.

The characters apart from _chromogenesis_, were very similar to one another on the whole, however, and agreed very well with the corresponding findings with _bacillus typhosus_ and _coli_. Reaction was without appreciable effect. Chromogenesis, on the other hand, varied considerably, and to a certain extent seemed to be attributable not to reaction, or other factors mentioned at the beginning of the chapter, but to the albuminous composition of the media, (including glycerine). Glycerine seemed to be a decisive factor in the production of a dark pigment, while meat extract was associated with the red-brown color, with the tendency toward formation of scarlet in the condensatio water.

As far as cultural features are concerned, the composition the presence or absence of certain definite substances- was of much greater importance than the actual reaction of the medium, within the limits studied in this work. These variations were modified to a certain extent by the tendency toward individual variations on the part of the organisms themselves.

Bacillus pyocyaneus. (Tables 25-30)

As before, it will be necessary to tabulate the findings upon the various media first, then discuss them with reference to the influence of reaction, composition of the medium, etc.

Agar 1; form of growth; filiform, 12; nodose,1; villous,4. elevation of growth; flat, 16; raised,1; topography; smooth,14; squamose, 3: optical character ; opaque,17: viscosity, negative,17.

Agar 2; form of growth; filiform, 16; echinate,1; lustre; shining,13; iridescent, 4: topography; smooth,13; squamose,4: optical character ; opaque,17; viscosity; negative,17.

Agar 3; form of growth; filiform, 13; echinate,1; beaded, 1; villous, 2: elevation; flat,17; lustre; shining,13; iridescent,4; topography; smooth,13; bullate,4;optical characters; opaque,17; viscosity, negative,17.

Agar 4; form of growth; filiform,15; echinate,2; elevation; flat,17; lustre; shining,15; iridescent,1; topography; smooth,15; squamose,2; optical characters; opaque,17.

Agar 7; form of growth; filiform,16; echinate,1; elevation; flat,17; lustre; shining,15; dull,1; iridescent,1; topography; smooth, 15; squamose,1; viscosity,negative,17.

Agar 8; form of growth; filiform,14; echinate,2; villous,1; elevation; flat,12; raised, 2; convex, 3; topography; smooth,15; squamose,1; rugose,1; lustre; shining,13; dull,1; iridescent, 3: optical characters; opaque,17;

Agar 10; form of growth; filiform, 17: elevation; flat, 16; raised.1; lustre; shining,15; iridescent,2; topography: smooth,14; squamose,2; rugose,1: optical characters; opaque,17; viscosity,17 negative.

Agar 11; form of growth filiform, 15; echinate,2; elevation; flat,16; raised,1; lustre; shining,13; iridescent, 4; topography; smooth,15; squamose,2; optical characters, opaque,17; viscosity; negative,17.

Agar 12; form of growth; filiform,17; elevation; flat,16; raised,1; lustre; shining,12; iridescent, 5; topography; smooth,14; squamose,3; optical characters; opaque; 17.

Agar 13; form of growth; filiform, 17; elevation; flat,16: raised,1; lustre; shining,14; iridescent, 3; topography; smooth,16; squamose,1; optical characters;opaque,17.

Agar 14; form of growth; filiform, 17; elevation; flat,17; lustre; shining, 12; iridescent,5; topography; smooth,16; squamose,1; optical characters; opaque,17; viscosity; negative, 17.

The results obtained with the organism under consideration are very interesting, and bring out some points which hitherto have been obscure.

The above table has been inserted to bring out these changes more readily.

<u>Bacillus pyocyaneus</u> produces both a pigment, pyocyanin, characteristic of the organism, and another pigment, soluble in the medium, a fluorescent pigment, which is common to a number of so-called fluorescent bacteria..The exact relationship between these pigments, their changes according to varying conditions, and the influence of various changes in reaction and composition of the media upon which they are grown are as yet not clearly understood.

Reference has been made to the recent investigations of Wassermann,(loc.cit.) who has done the best and most complete work up to date upon many of these points. His results have been considered in Part 11;

Because of the great interest attaching to these points, great care has been taken to check the results, which are in many ways different from those previously presented. Additional samples of agar have been made, by the <u>same</u> observers and under the <u>same</u> conditions, from the same

materials, and utilized in this connection, and [illegible], every precaution has been taken to insure uniformity of results.

The cultural characteristics, exclusive of chromogenesis and fluorescence, are of little interest, and will be discussed first, indicating any influence which reaction, concentration or composition of the medium may have upon them. Chromogenesis and fluorescence, because of their importance as diagnostic characteristics of *Bacillus pyocyaneus*, will be discussed later, and separately.

Cultural characteristics of agar slants of *Bacillus pyocyaneus*.

Agar 1; there are two well defined types of growth upon this agar; those having an almost invisible, shining growth, and those having a more marked, silvery or iridescent lustre. Almost invariably the organisms grow as non-characteristic, filamentous colonies, flat, smooth. There is a slight tendency for some to spread, but this is not marked. The growth was luxuriant upon this medium. Agar 2; agar 3: growth filiform, without any decided tendency to spread. The same two types appear upon this medium. In some few instances, the silvery lustre was not spread

over the whole growth, but was confined to patches. There was no distinct relation between the thickness of the layer of bacteria, proximity to the condensation water, and the appearance of this metallic lustre. Furthermore the phenomenon was not associated with drying, and the peculiar distribution is one of the peculiar variations to which certain bacteria are prone. The growth was moderately luxuriant, flat and as a rule smooth, although a few cultures showed a tendency to form a wrinkled surface. This feature, however, was an individual peculiarity upon the part of certain strains of bacteria rather than any inherent property of the media as is shown by the fact that the same variation occurred much more frequently in individual cultures than upon specific media. Agar 4; the organisms grew luxuriantly upon this medium; more so than upon any of the preceeding. This has been the case almost invariably so far. The cultures of *Bacillus pyocyaneus* upon this medium did not have the same tendency to spread as was the case with the previous organisms studied. The growths were filiform, flat, smooth, and opaque. The silvery lustre, mentioned above, was less conspicuously developed than with other agars. In only two

Ones did it appear to any considerable extent.

Agar 7; growth moderately luxuriant, with a tendency to spread, and almost invariably shining; the metallic lustre when present, did not cover the whole surface, but appeared in patches, which were more or less diffuse. The cultures showed a tendency to form thick, raised growths in contrast to the flat growths noticed hitherto. The colonies were in the main smooth, although some of the thicker growths were slightly wrinkled.

Agar 8. Growth luxuriant, filiform, slightly spreading, and almost invariably shining; the metallic lustre was when present diffuse, forming only a small part of the surface appearance. The growth upon this agar was the most constant observed so far.

Agar 11; this agar showed great variations of growth-forms. Particularly variable was the lustre; the two types, shining and metallic, occurred apparently indiscriminately upon the same culture. There was no regularity about this characteristic. The general tendency was towards the formation of a filiform growth, flat, smooth, and opaque.

Agar 12;,13,14. These agars were almost identical in their characteristics; luxuriant, filiform, flat, shining, smooth

surfaces.

In general, the growth upon any of the agars was not very unlike any other agars, and one would not have very much difficulty in recognizing, so far as the morphology of the organisms was concerned, the various cultures as being those of the same organism.

Chromogenesis and Fluorescence.

These two characteristics varied widely: in fact one could not identify Bacillus pyocyaneus upon certain of the media by its chromogenic and fluorescent properties. The general appearance of the organisms upon different agars will be discussed first. The apparent cause of the variations in chromogenesis and fluorescence will be discussed later.

Agar 1; surface growth usually dark green (pyocyanin). Certain cultures showed no pigment at all. Fluorescence was only slightly developed.

Agar 2;, agar 3; both pyocyanin and fluorescent pigment were poorly developed upon these semi-solid agars. Many cultures produced neither pigment nor well marked fluorescence. The pyocyanin, when present, had usually a well marked red-brown color.

Agar 4 was unique- the insoluble pigment, pyocyanin, was almost invariably absent, while the soluble, fluorescent, pigment was extraordinarily well developed. This phenomenon seemed to be constant for every culture examined.

Agar 7; the fluorescent pigment was absent as a rule, when pyocyaneus was grown upon this agar. The insoluble pyocyanin showed great variations in color, varying from a rather light yellow to red-brown.

Agar 8; Again fluorescence is almost always absent from this medium, while the pyocyanin varied greatly in color. In a very few instances, both the fluorescent and insoluble pigments were absent.

Agar 10; The fluorescent pigment was absent, while the pyocyanin showed colors varying from red, brown to even blue.

Agar 11; both pigments were very variable upon this medium. No general tendencies toward the production of definitely colored products of either product were noted.

Agars 12,13,14; these agars varied slightly from one another. The usual tendency was to produce a soluble pigment, together with a slight amount of pyocyanin.

Influence of the various chemical and physical properties of the media upon the growth of Bacillus pyocyaneus.

Influence of reaction.

The study of the previously considered bacteria, _Bacillus coli_, _typhosus_ and _prodigiosus_, has demonstrated that, with the conditions under which they were grown, the reaction of the various media had practically no influence upon the morphology of the growth or the production of pigment.

While there were the greatest variations in the color of the pigment produced by the prodigiosus cultures, and considerable in the fluorescence of _Bacillus pyocyaneus_, the changes are of such a character that they cannot be attributed to the reaction of the medium, because the variations were as great upon media having the same reaction as upon those having widely different reactions.

Influence of concentration of the medium.

No effect was noted when the concentration of agar ranged from 0.5 to 1.5 per cent. In general, the fluorescence was most marked in agars having less concentration, than in those in which the percentage of agar is greater. Apparently, too, the thoroughness with which agar is dissolved is not without influence upon the final result. The lesser concentrations not only seem to favor the so-

lution of soluble pigments, but that growths are apt to be more spreading with the lower amounts of agar in the medium.

Influence of temperature and moisture;

Temperature had a marked effect upon the pigment production of _Bacillus pyocyaneus_ particularly when the cultures were kept in the thermostat in a dry atmosphere. The changes in the pyocyanin, during which time the various colored products are formed, take place more rapidly at 37°; in fact, when some of the cultures were grown at 20° the pyocyanin did not change from the characteristic blue-green to the oxidation products, the reds and browns.

Cultures of _Bacillus pyocyaneus_ kept in a moist atmosphere formed the characteristic pigment much more abundantly than similar cultures kept in a perfectly dry atmosphere. This feature was more marked at 37° than at 20°. The formation of oxidation products of pyocyanin was more marked in a moist atmosphere than in a dry environment; this is probably due to the fact that the cultures did not dry up as rapidly. In moist atmospheres the cultures remained alive surprisingly long times, considering the fact that pyocyanin is a bacteriolysin.

Influence of certain chemical constituents.

It has been shown that the variations, particularly in pigment production, cannot well be explained upon the basis of change in reaction of the medium, etc. Change in the composition of the medium, however, seems to have a decided effect. It will be well to begin with the simplest agar, and trace the changes with increasing complexity of composition.

Agar 7, containing peptone and agar. An examination of the tables will show that fluorescence is practically absent upon this medium; on the other hand the pyocyanin is very well developed The various oxidization products of the pyocyanin are even more marked than the blue-green color of the pigment itself. Several stages in the process, light yellow, green, dark brown, olive green and purple were noted. It is extremely interesting to compare these findings with those of other work upon the same subject.

[illegible] (loc. cit) found that the pyocyanin dissolved in chloroform giving a red color, when such a solution is treated with hydrochloric acid; a yellowish color with reducing agents and a blue color with alkalis.

Jordan (Cent.f. Bakteriologie, Bd.15) found that

the red pigment is a [illegible] produced by some change in the [illegible].

It has been shown in the tables that in the same agar tubes, made at the same time, under the same conditions, having essentially the same composition, the above changes may take place side by side. The only demonstrable difference is the difference in strain of the organism. Even this is not always constant.

It is quite unlikely that hydrochloric or in f ct any free mineral acid is present in the cultures, yet one finds the yellow color present frequently. Again, there cannot be much free alkali present, yet one finds the blue color appearing. Either we have two series of changes, whose results are the same, one caused by the action of acid, alkali or reducing substances, while the other is caused by the organisms themselves, or else we are compelled to believe the strain of the organism has much to do with these phenomena. The latter conclusion is logical in view of the individual variations noted in the tables.

Agars 2,[illegible],11,12,13,14. composed of meat juice, peptone and agar. These agars were made by different individuals,

nos. 2,11; 3,12 of the same individuals at different times. The most noticeable result one sees by glancing at the tables is that the pigment-pyocyanin- is not well developed. There is a slight amount produced by certain <u>strains</u> of <u>Bacillus pyocyaneus</u>, but this is not by any means the rule. One may fairly assume that these samples of agar are not rich in those materials from which the organism may derive products necessary for the production of the characteristic pigment of the organism. As a rule, when this pigment was produced, the oxidation products, red-brown particularly, were the ones most frequently met with. This coloring material was originally described as the third of the pigments characteristic of pyocyaneus, but we now know that this is a mistake, and that it merely represents a stage in the gradual oxidation of pyocyanin. Fluorescence was very well developped, although the various strains of the organisms, not the character of the agar were the exciting cause determining the intensity of the color. It is difficult to account for the differences of [illegible] in these agars, differences which seem to be rather characteristic of the various samples of the media; it is to be suggested that again we have a slight indication of a 'personal factor' in the making of each

medium. This is rendered more probable because of the fact that they were made at the same time, from the same lot of materials, and the reaction varied from [illegible] to [illegible], a very slight difference in reaction. Agars [illegible] and 11 were characterized by the preponderance of the red-brown modification of pyocyanin, while 3 and 12 were noteworthy because of the excess of cultures having the green pigment. These samples were made by individuals skilled in making media, and although the changes noted above are slight, they are quite constant. As this phenomenon has happened again and again during the progress of the work, it cannot be accidental, and the personal factor is the logical interpretation of the phenomena. The personal factor in media making as well as in making observations, is another variable which one must take into consideration in bacterial work.

Agars 1 and 8; agar 1, composed of malt extract, glycerine and peptone; agar 8, peptone, glycerine and agar. The glycerine had a tendency to darken the color of the pigment produced by Bacillus prodigiosus, and the same general tendency obtained with cultures of Bacillus pyocyaneus. The color of the pyocyanin was darker when media containing-

glycerine than upon any other media. One is not justified in generalizing upon so small an amount of evidence, particularly when one is dealing with living things, but the results have been so consistent during the progress of this work that they can hardly be looked upon as accidental, and must have some weight. Agar 1 was further characterized by the production of a peculiar, metallic lustre which was entirely lacking in agar 0. It will be remembered that those media containing meat extract gave this appearance, while other agars gave it slightly or not at all.

Agar 4, composed of meat juice, peptone and agar. We have seen that peptone agar and meat extract-peptone agar tend toward the formation of pyocyanin. That glycerine has a distinct tendency to produce a darker pigment, without the production of considerable amounts of fluorescent pigment. The latter medium is apt to be characterized by a greater amount of fluorescent pigment than the peptone agar. The great characteristic of meat juice agar is the formation of a very decided fluorescent pigment, and the relative absence of pyocyanin. This was very definite and more striking than any other characteristic considered.

so far. This can hardly be due to the reaction, since media having the same composition, but a neutral reaction gave precisely the same results. The probability that the reaction has practically nothing to do with the fluorescence is rendered all the more probable by the fact that certain of the neutral meat extract agars showed a certain amount of fluorescence when inoculated with pyocyaneus.

It is difficult to explain the absence of the characteristic pigment, pyocyanin, upon meat juice agar, while the non-characteristic pigment, fluorescein, is present, when the only change is the substitution of meat juice for meat extract, and when the serum medium showed practically the reverse.

A priori one would expect there would be less difference between meat juice and blood serum of the same animal, than between meat juice and meat extract.

The whole investigation, while done on a rather large scale, is full of these perplexing questions, and much more extended and elaborate investigations are necessary before one can definitely settle these points.

The results presented, while significant, cannot be regarded as final, and must be corroborated by investigators

using the same general methods, but carried out in greater numbers. Enough has been presented, however, to show that the work of the past has been done upon much too small a scale, and that coordinated, systematic investigations are necessary to settle finally the relation between variations in media making, the 'personal factor' and the extent to which one must rely upon cultural as opposed to biochemical characteristics for the classification of bacteria.

Summary.

The results of the influence of reaction, concentration factor, moisture and chemical composition of media upon the form of growth of organisms have been quite thoroughly discussed at the end of each section, and for each of the four organisms studied.

We have seen that colony formation upon plates depends upon conditions such that the variations of an organism upon a given plate are quite as great with the same species and same medium as would be the case with the same species of organism upon different plates, grown in media containing the usual ingredients, and of a reaction and concentration such that bacteria can reasonably be expected to grow and produce their characteristic colonies.

This has been the experience of bacteriologists, and needs no further comment at this time. In a given plate one involuntarily chooses a typical (average) colony for description; the color may not be distinctive, and resemble colonies of other bacteria, yet this is the basis from which all species work must start, since bacteriologists must depend upon plating methods for the separation of bacteria, and their isolation in pure cultures. Upon the gelatin stab, as a rule, nothing is constant or important except the liquefaction or non-liquefaction of gelatin.

Agar slants, with the non-chromogenic, non-characteristic bacteria, are influenced by the concentration of the agar to a certain extent, and the luxuriance of growth is varied by the kind of proteid present, meat juice being very favorable in general for bacterial growth.

The chromogenic bacteria react to a greater extent to changes in the albuminous content, and also to certain chemicals, glycerine notably. The latter substance has a tendency to cause a general darkening of the color of the pigment. The morphology of the growth is not changed by these substances. The albuminous substances too have a decided action; this cannot be specifically stated a

priori, but must be found out by actual experiment.

The proteolytic and prodigiosus cultures have been studied in considerable detail, and the differences noted above; it seems very probable that like changes will be found to occur with other chromogenic bacteria as one or more of substances be used as the basis of the medium upon which they are grown.

Blood serum seems to be quite as valuable for furnishing bacterial nutriment as meat extract, and is not open to the objection that the inorganic salt content is so variable

These facts have a bearing upon descriptive bacteriology; descriptions of chromogenic bacteria must be made with much greater attention to details if such determinations are to be useful and reliable to bacteriologists. Not only must the variations occurring upon a given medium be discussed, but the composition of the medium, particularly with reference to the composition of the proteid material be included, so that one may have some basis upon which to repeat the observations, and obtain concordant results.

Section 4; Interpretation of the same cultural phenomena by different observers; the personal factor o [illegible]-

ural interpretation.

The 'personal factor' in interpreting Cultural Characters of Bacteria.

As a basis for this study, twenty five students were placed at my disposal through the kindness of Dr. Welch and Dr. Ford.

These men made systematic study of thirty (30) organisms using the numerical method of tabulation mentioned elsewhere, and working in groups of three men each. Each man made separate tabulations of the characters of these bacteria, and each group of three made records of the same cultures.

The writer made observations on each set of cultures, and tabulated his results together with those of the students in the accompanying tables.

For convenience of identification, the students are numbered 1-25, and after each three observations, my own follow in red ink. Since all four results were made from the same set of cultures, one may compare the differences in interpretation by merely noting the different numbers for the same characteristic.

It is obviously impossible to describe exactly each personal peculiarity (personal factor) in interpreting

these results, but a few general statements such as the [illegible] which will indicate the principal points brought out by this investigation.

1. There is a 'personal factor' in making observations upon the same set of bacterial cultures by different observers.

2. These variations diminish as the cultures approach more nearly a definite type, (as a typical filiform growth upon agar, for example,) and increase as the characteristic becomes nearer the mean between two types, (as between a filiform and echinate growth for example).

3; The variations in cultural characteristics observed are similar in nature to those found in the first portions of this investigation; variations in intensity, but not of kind.

The reaction of media, and other similar characteristics gave precisely the same results as similar experiments in the preceding portion of this chapter.

4. Cultural characters are unsuitable for primary division of bacteria into groups.

The following pages are a summary of the characteristics found in this research with thirty organisms.

Bacillus prodigiosus. No. 1. 23 observations.

Agar slant cultures.

form filiform, 21. spreading, 2.
elevation. flat, 20. [illegible], 3.
lustre. shining, 23.
chromogenesis. red, [illegible]. red-brown, 4. light red; 15.
red-orange. 1.
topography. smooth, 3; contoured, 1. 0, 3.
optical characters; translucent, 2; opaque. 21.
viscosity. +, [illegible]. -, 14.
Gelatin stab.
form of liquefaction. saccate, 14; infundibuliform, 1;
stratiform, 8.
line growth. filiform, 4; no growth, 1[illegible].
surface growth. pellicle, 7; no growth; 16.
Broth.
pellicle. +,7. -,16.
turbidity: slight, non-characteristic; 2. decided, non-characteristic, 18. no turbidity, 3.
Potato.
form filiform,15; [illegible],2; beaded, 2; echinate, 2,
spreading, 2.
elevation. flat, 11; raised, 5; convex, 5; [illegible],1;
umbilicate, 1.
lustre. shining, 20; waxy, 2; iridescent,1.
chromogenesis. red, 2; red-brown, 1. red-orange, [illegible].
light red, 13, brown-red, 1.
potato discolored. +, 18; -, 3; 0, 2.
Gelatin colony.
form. round, 17. 0, 6.
edge. entire, 13. serrated, [illegible], erose, 1; 0, 6.
elevation. flat, 4; raised,11; umbonate,1; convex,1;
0, 6.
internal structure. grumose, 16; 0, 7.
chromogenesis; red-brown, [illegible]; light red,[illegible]; red-orange,[illegible];
0,6.
optical characters. translucent, 1; opaque, 15; 0,7.
Agar colony.
form. round,10; conglomerate,4;rosulate,1;0,3.
elevation. flat,1[illegible]; raised,4; umbonate,4; 0,3.
edge. entire,11; raised, 4; lobed, 3; 0,3.

Bacillus prodigiosus. continued.

Agar colony.

internal structure. grumose, 13; homogeneous, 4; clouded, 3; 0, 3.

Milk.

coagulated. +, 20; -, 3.

liquefaction. +, 20. -, 3.

reaction. acid, 19; alkaline, 2; 0, 2.

Bacillus indicus. No. 2. 23 observations.

Agar slant.

form. filiform, 18; echinate, 2; spreading, 3.

elevation. flat, 11; raised, 2; convex, 4; effuse, 2; capitate, 4.

lustre. shining, 18; dull, 4; waxy, 1.

chromogenesis. red, 11; reddish, 5; red-brown, 1; gray, 1; light red, 4; orange, 1.

topography. smooth, 14; undulate, 1; rugose, [illegible]; contoured, [illegible].

optical characters. oleaginous, 1; translucent, 4; cretaceous, [illegible]; opaque, 16.

Gelatin stab.

form of liquefaction. crateriform, [illegible]; infundibuliform, 10; stratiform, 5; saccate, 3. 0, 2.

line growth. filiform, 7; -, 16.

surface growth. pellicle, 16. -, 7.

Broth.

pellicle, +, 9; -, 14.

turbidity. non-characteristic, 19. none, 4.

Potato.

form. filiform, 13; nodose, 2; beaded, [illegible]; echinate, [illegible]. spreading, 2.

elevation. flat, [illegible]; raised, 11; convex, 8; capitate, 1; umbonate, 1.

lustre. shining, 15; dull, 3; waxy, [illegible].

chromogenesis. brownish, 2; reddish, 5; red-orange, 5; red-brown; 7; white, 1; blue, 1; gray, 1.

potato discolored. +, 16. -, [illegible]. 0, 1.

Gelatin colony.

form. round, 9; conglomerate, 1. 0, 13.

elevation. flat, flat, 2; raised, 1; convex, 5; papillate, 1; 0, 14.

edge. entire, 7; 0, 16.

internal structure. hyaline, [illegible]; finely granular, 3. grumose, 4; 0, 14.

chromogenesis. gray 2; red 3; red-brown 2; light red [illegible];
 0, 14.
optical characters. opaque 7; paraffinous 2; 0,14.

Agar colony.
form. round 18; conglomerate 3; 0, [illegible].
elevation. flat 1; raised 4; convex 1[illegible]; 0,2.
edge. entire 15; waved 4; auriculate 2; ciliate 1;
 0,1.
internal structure. amorphous 6; granular 7; grumose 5
 clouded 3; 0, [illegible].

Milk.
coagulated + 15; -,5; 0,2.
liquefaction. + 1[illegible]; -, [illegible]; 0,[illegible].
reaction. acid 1[illegible]; alkaline 3 0,2.

Bacillus janthinus No.3. 23 observations.

Agar slant.
form. filiform 19; echinate 3; 0, 1.
elevation. flat, [illegible]; effuse, [illegible]; raised, 1[illegible]; convex, 2;
lustre. shining; 18. waxy, 5;
chromogenesis. blue, 8; purple, 15.
topography. smooth 21; contoured, 2.
optical characters. nacreous, [illegible]; opaque, 20.
viscosity. +, 5; -,1[illegible].

Gelatin stab.
liquefaction. form- saccate, 10; infundibuliform 10,
 napiform, 3;
line growth. filiform, 7; no growth, 16.
surface growth. flat, 23.

Broth.
pellicle. +, 5; no pellicle, 18.
turbidity. clears up on standing, 1, clouded, 2[illegible].
sediment, non-characteristic, 23.

Potato.
form. filiform. 18; no growth, 5.
elevation. flat, 7; raised, 10; convex, 3; umbilicate, [illegible].
lustre. shining, 17; waxy, 6.
chromogenesis. brown-red, 1; red-orange, 2; blue, 3;
 purple, 12.
potato discolored. +, 16; no discoloration, 7.

Gelatin colony.
form. round, 18. no colony grown, 5.

edge. entire, 17; erose, 1; 0, 5.
elevation. flat, 1; convex, [illegible]; umbilicate, 15. 0, [illegible].
internal structure. homogeneous, 4; granular, [illegible];
grumose, 12; 0, 5.
chromogenesis. yellow, 2; [illegible], 3; [illegible], 13; 0, 5.
optical characters. opaque, 1[illegible]; 0, 13.

Agar colony.
form. round, 21; 0, 2.
elevation. flat, 3; effuse, 3; raised, 12; convex, 3
0, 2.
edge. entire, 21; 0, 2.
internal structure. homogeneous, 4; granular, [illegible]; grumose, 1[illegible]
0, 5.

Milk.
coagulated. 14; -, [illegible].
liquefaction. +, 7; -, 15.
reaction. acid, 11; alkaline, 1.

Sarcina lutea. No. 4: 23 observations.

Agar slant. filiform 15; [illegible], 3; [illegible], 1; spreading, 1.
elevation. raised, 13; convex, 10.
lustre. shining, 23.
chromogenesis. yellow, 3; yellowish, 4; yellow-brown, 15
orange, 1.
topography. smooth, 23.
optical characters. [illegible], 3; butyrous, 2; opaque, 18.
viscosity. +, 3; -, 20.

Gelatin stab.
liquefaction, saccate, 2; no liquefaction, 21.
line growth. filiform, 15; beaded, 3; echinate, 1; villous, [illegible].
surface growth. flat, 3; effuse, 4; convex, [illegible]; umbil-
icate, 3- no growth, 5.

Broth.
pellicle. no pellicle, 23.
turbidity. clears up on standing, 10: non-character-
istic, 11. no turbidity, 2;
sediment. 17. no sediment, 6.

Potato.
form. filiform, 15; beaded, [illegible]; 0, 4.
elevation. flat, 5; raised, 5; convex, 9; 0, 4.
lustre. shining, 15; waxy, 4; 0, 4.
chromogenesis. brownish, [illegible]; purple, 1; yellow, [illegible]; yel-
lowish, [illegible]; yellow-brown, [illegible]; 0, 4.
potato discolored. none.

Gelatin colony.
form. round, 1[illegible] [illegible], 10.
elevation. raised, 11; convex, [illegible], [illegible], 1[illegible].
edge, entire, 13; [illegible], [illegible].
internal structure. homogeneous, 1[illegible]; granular, [illegible]; [illegible], 11.
chromogenesis. orange, 1; orange-brown, [illegible]; yellow, [illegible];
yellowish, 1; [illegible], 10.
optical characters. opaque, 1[illegible]. 0, 11.
Agar colony. round, 22; 0, 1.
elevation. flat, [illegible]; raised, [illegible]; convex, [illegible]; umbilicate, [illegible]
0, [illegible].
edge. entire, [illegible]; 0, 1.
internal structure. homogeneous, 14; granular, [illegible]; [illegible], 1.
Milk.
coagulated. [illegible]. no coagulation, 17.
liquefaction. negative, [illegible].
reaction. acid, [illegible]; alkaline, 13; no culture, 1.

Bacillus rosaceous metalloides. No.[illegible], 22 observations.

Agar slant.
form. filiform. 1[illegible]; echinulate, [illegible].
elevation. flat, [illegible]; effuse, 1; raised, [illegible]; convex, 4; umbonate, [illegible].
lustre. shining, 1[illegible]; waxy, 3.
chromogenesis. reddish, 1; orange, 1[illegible]; light orange, [illegible].
optical characters. oleaginous, [illegible]; outgrown, [illegible]; opaque, 1[illegible].
topography. smooth, 17; punctate, 3; contoured, [illegible].
viscosity. +, 5; -, 17.
Gelatin stab.
liquefaction. form. crateriform, [illegible]; stratiform, 1; 0, 1[illegible].
line growth. filiform, 1[illegible]; echinulate, [illegible].
surface, flat, 1[illegible]. no growth, [illegible].
Broth.
pellicle. +, 6. -, 16.
turbidity non-characteristic. 22.
sediment. non-characteristic, 16. none, 6.
Gelatin colony.
form. round, 12; no colony, 10.
elevation. raised, [illegible]; convex, [illegible]; [illegible], 1[illegible].
edge. entire, 1[illegible]; [illegible], 1[illegible].
internal structure. homogeneous, 1; granular, [illegible]; [illegible];
gyrose, 1[illegible]; [illegible], 10.
chromogenesis. orange, [illegible]; orange-brown, [illegible]. 0, 1[illegible].
optical character. outgrown, [illegible]; opaque, [illegible]; 0, 1[illegible].

Agar colony.
form. round, [illegible].
elevation. flat,[illegible]; raised,[illegible]; convex,1; umbilicate,[illegible].
edge. entire,[illegible].
internal structure. homogeneous,2; granular,1[illegible]; grumose [illegible];
gyrose,3; 0,1.
Milk.
coagulation. +,[illegible]. -,[illegible].
liquefaction. +,[illegible]; -,1.
reaction. acid,15. alkaline,[illegible].

Bacillus cyaneus. No. [illegible]. 22 observations.
Agar slant.
form. filiform,1[illegible]; nodose,4; echinulate,5.
elevation. flat,1; raised,11; convex,10.
lustre. shining,20; iridescent,[illegible].
chromogenesis. yellowish, 1, yellow-red,7; green-yellow,
1[illegible], blue,[illegible]; greenish,2.
optical character. [illegible], 1; opaque,21.
topography. smooth,22.
viscosity. negative, 22.
Gelatin stab.
form of liquefaction. crateriform,21; 0,1.
line growth. filiform,[illegible]; beaded,[illegible]; 0,5. echinulate,2.
surface growth. flat,4; no growth,18.
Broth.
pellicle,1, no pellicle,21.
turbidity. non-characteristic, [illegible].
sediment, non-characteristic, 22.
Potato.
form. filiform,14; nodose,1; echinulate,2; spreading,1.
no growth, 4.
elevation. flat,1; raised,[illegible]; convex,1[illegible]; [illegible],3;
no growth,4.
lustre. shining,7; dull,5; wax ,5.
chromogenesis. yellow,[illegible]; light yellow,1; yellow-brown
[illegible]; green-red,4; green-orange,7; purple,[illegible].
no growth, 4.
potato discolored. +, 18. no growth,4.
Gelatin colony.
form. round, 21; no colony, 1.

elevation. flat, 1; convex,8; 0,11.
edge, entire,11; 0,11.
internal structure. granular, [illegible]; 0,14.
chromogenesis. red-yellow,[illegible]; yellowish,4; [illegible],[illegible];
green-brown,[illegible]; 0,11.
optical characters. opaque,11; 0,11.
Agar colony. form; round, [illegible]; 0,1.
elevation. flat, 21.
edge. entire, 1; lobate, [illegible]; ciliate,8; serrate,1[illegible];
internal structure. granular,[illegible]; grumose,[illegible]; gyrose,1.
Milk.
coagulated. +,12; 0,10.
liquefaction.+,11; 0,11.
reaction. acid,16; alkaline,6.

Bacillus coli. No.7. 34 observations.

Agar slant.
form. filiform, 4; echinulate,[illegible]; villous,10,arborescent,14.
elevation. flat,1[illegible]. effuse,1;raised,[illegible];[illegible],6
umbonate,2.
lustre.shining,[illegible]; dull,4; waxy,[illegible].
chromogenesis.white,1;gray,1[illegible];brown,7;yellowish,[illegible].
topography.smooth,26; rugose,1;contoured,7.
optical characters.opalescent,2[illegible]; opaque,1[illegible].
viscosity.+,22. -,12.
Gelatin stab.
liquefaction.negative,34.
line growth.filiform,7; nodose,1; beaded,3; echinate,3.
surface growth.flat, 1; raised,4; convex,4; effuse,1.
spreading,1, 0.3.
Broth.
pellicle.+,3; 0,31.
turbidity.non-characteristic,[illegible] 34.
sediment.non-characteristic, 34.
Potato.
form.filiform,25; nodose,[illegible]; echinate,4; villous,1;
spreading,1.
elevation.flat,[illegible]; raised,16; convex,15; umbonate,1.
lustre.gray,2; brown,1[illegible]; brown-red,7; yellowish,[illegible];
yellow-brown, 10.
potato discoloration. +,[illegible]. -,[illegible].
Gelatin colony.
form.round,[illegible]; amoeboid,[illegible];0,1.
elevation.flat,1[illegible]; effuse,1; raised,[illegible]; convex,[illegible];
umbonate,[illegible]; 0,1.

edge.entire,22; waved,7; serrated,4;0,1.
internal structure.homogeneous,11; granular,14; grumose,6; clouded,1; gyrose,1; 0,1.
chromogenesis.white,6; gray,[illegible]; brown,2; yellow,[illegible]. bluish,2; 0,1.
optical character.opalescent,14; nacreous,7; translucent,1; opaque,5; cereceous,1;parafinous,2 0,4.
Agar colony.
form. round, 34.
elevation.flat,16; raised,5; convex,10; umbilicate,3.
edge. entire, 21; waved,9; auriculate,1; erose,3 .
internal structure.homogeneous,3; granular,11; grumose, 13; gyrose,4.
Milk.
coagulated. +,34.
liquefaction.+,13. -,21.
reaction. acid, 34.

Bacillus typhosus. No.8. 34 observations.
Agar slant.
form.filiform, 19; echinate,11; villous,5.
elevation.flat,20; effused,1; raised,11;convex,2;
lustre. shining,31; dull,3.
chromogenesis. white,4; gray,24; brown,5; yellow,1.
topography.smooth,34.
optical character. transparent,2; gray,1; opalescent 27 opaque,3; nacreous,1.
viscosity. -,34.
Gelatin stab.
liquefaction. negative,34.
line growth.filiform,30; nodose,1; beaded,1; villous,1; plumose,1.
surface growth.flat,[illegible]; effuse,1; raised,1;convex,3. no growth,[illegible].
Broth.
pellicle. +,1. 0,33.
turbidity.non-characteristic,34.
sediment,non-characteristic. 34.
Potato.
form.filiform,30; echinate,[illegible]; spreading,1.
elevation.flat,9; raised,15; convex,1[illegible]; capitate,[illegible]; umbilicate,1
lustre.shining,28; dull,2; waxy,4;

chromogenesis. grey,8; brown,[illegible]; brown-red,[illegible]; yellowish,[illegible]; yellow,[illegible].

potato discolored, 23. not discolored,11.

Gelatin colony.

form. round,21; no colony, 13.

elevation. flat,5; convex,16; 0,13.

edge. entire,21; 0,13.

internal structure, homogeneous, 6; granular, 13; grumose 2; 0,13.

chromogenesis. white, 1; grey,14; brownish,1; yellowish,2; bluish,3; 0,13.

optical characters. transparent,1; translucent,7; [illegible], 1; opalescent,12; 0,13.

Agar colony.

form. round, 34.

elevation. flat, 6; raised, 1; convex,27;

edge. entire, 28; lobed, 2; auriculate,1; [illegible].

internal structure. homogeneous, 8; granular,18. areolate,1; grumose,7.

Milk.

coagulated, negative,34.

liquefaction. negative,34.

reaction. acid,16; alkaline,6; amphoteric,[illegible]; terminal alkalinity, 12.

Micrococcus albus. Gr. 9, 33 observations.

Agar slant.

form. filiform, 25; beaded,4; echinate,3; acicular,1.

elevation. flat,8; raised, 16; convex,6; umbilicate,2; umbonate,1.

luster. shining,26; dull,2; waxy,5.

chromogenesis. white,12; grey,18; brownish,2; orange-yellow,1.

topography. smooth,32. papillate,1.

optical characters. translucent,3; opalescent,6; opaque,[illegible]; nacreous,1; oleaginous,1.

viscosity. negative,33.

Gelatin stab.

liquefaction, form. crateriform,3; saccate,[illegible]; negative,[illegible].

line growth. filiform,17; echinate,1; 0,15.

surface growth. flat,4; negative,29.

Broth.

pellicle. +,3. 0,30.

turbidity. clears up on standing, 3; no turbidity. [illegible].
sediment. non-characteristic. 33.

Potato.

form. filiform, 29; beaded, 3; echinate, 1;
elevation. flat, 3; effuse, [illegible]; raised, [illegible]; convex, [illegible]; capitate, [illegible]; umbonate, [illegible].
lustre. shining, 24; dull, [illegible]; waxy, [illegible].
chromogenesis. white, 11; gray, 1[illegible]; brownish, [illegible]; brown, 1, brown-yellow, 1; yellowish, 2; yellow-brown, [illegible].
potato discolored. +, [illegible]; -, [illegible].

Gelatin colony.

form. round, 17; 0, 16.
elevation. flat, [illegible]; raised, [illegible]; convex, 3; capitate, [illegible]; umbilicate, 1; 0, 16.
edge. entire, 16; ciliate, 1; 0, 1[illegible].
internal structure, granular, [illegible]; grumose, [illegible]; gyrose, 3.
chromogenesis. white, [illegible]; gray, 7; reddish, 1; 0, 1[illegible].
optical characters. translucent, 4; opalescent, 3; opaque 10; 0, 16.

Agar colony. form. round, 2[illegible]; 0, 7.

elevation. flat, 2; raised, 9; convex, 10; capitate, 1; umbilicate, 2; 0, 7.
edge. entire, 26; 0, [illegible].
internal structure. homogeneous, 1; granular, 1[illegible]; grumose, 7; clouded, [illegible]; 0, 7.

Milk.

coagulated. +, 14: 0, 19.
liquefaction. negative, 33.
reaction. acid, 27; no change, 6.

Micrococcus (streptococcus) pyogenes. No. 10.
33 observations.

Agar slant.

form. filiform, 9; nodose, [illegible]; beaded, 14. [illegible], 4; spreading, 3.
elevation. flat, 1[illegible]; effuse, 4; raised, [illegible]; umbonate, 13.
lustre. shining, [illegible]; dull, [illegible]; waxy, 2.
chromogenesis. white, [illegible]; gray, 2[illegible]; brownish, 1
topography. smooth, 21; papillate, 3; rugose, 1.
optical characters. transparent, [illegible]; vitreous, 1; paraffinous, 3; translucent, 16; opaque, 5; opalescent, 1; sebaceous, 1; butyrous, 1.
viscosity. negative, [illegible]3.

Gelatin stab.

liquefaction. 35 negative.
line growth. filiform, 3[illegible]; beaded, 3; 0, [illegible].
surface growth. flat, 1; 0, 3[illegible].
Broth.
pellicle. none, 3[illegible].
turbidity. clear up on standing, 1[illegible]; none, 17.
sediment. non-characteristic, [illegible]; none, 3.
Potato.
form. filiform, 1; no growth, 3[illegible].
elevation. flat, 1; 0, 3[illegible].
lustre. shining, 1; 0, [illegible].
chromogenesis. white, 1; 0, [illegible].
potato discolored, 1; 0, [illegible].
Gelatin colony.
form. round, [illegible]; 0, 11.
elevation. flat, 3; raised, 3; convex, 8; [illegible], [illegible];
umbilicate, 2; 0, 11.
edge. entire, 19; waved, 1; lobed, 2; 0, 11.
internal structure. homogeneous, 1; granular, 1[illegible]; alveolate. 1;
grumose, 5; 0, 11.
chromogenesis. white, 1; gray, 14; brownish, [illegible]; red-brown, [illegible];
yellow, 1; orange, 1; 0, 11.
optical characters. transparent, 1; translucent, [illegible];
opalescent, [illegible]; cut-glass, 1[illegible]; 0, 11.
Agar colony.
form. round, 3[illegible].
elevation. flat, 1[illegible]; raised, [illegible]; convex, [illegible]; umbilicate, [illegible];
[illegible], 1.
edge. entire, 33.
internal structure. homogeneous, [illegible]; granular, 14; grumose 1[illegible].
Milk.
coagulated, 1, no coagulation, 3[illegible].
liquefaction. negative, 3[illegible].
reaction. acid, 3[illegible]. no change, 1.

Micrococcus [illegible]. No. 11. 34 observations.
Agar slant.
form. filiform, 2[illegible]; villous, 9.
elevation. flat, [illegible]; effuse, 3; raised, 1; umbonate, 1;
lustre. shining, [illegible]; dull, [illegible]; waxy, [illegible].
chromogenesis. white, 8; gray, [illegible]; brownish, [illegible].

topography. smooth,30; [illegible],1; rugose,3.
optical characters. transparent,11; vitreous,1; translucent,10; paraffinous,4; opalescent,5. nacreous,1; opaque,1.oleaginous,1.

Gelatin stab.
liquefaction. crateriform,[illegible]; saccate,[illegible]; infundibuliform,[illegible] negative,14.
line of growth. filiform,23; nodose,1; beaded,10.
surface growth. flat,3; umbonate,1; 0,25.

Broth.
pellicle. negative,34.
turbidity. clears up on standing,10; noncharacteristic. 16; 0,8.
sediment. non-characteristic,34.

Potato.
form. filiform,18; nodose,1; beaded,10; echinulate,[illegible]; spreading,3.
elevation. flat,[illegible]; raised,[illegible]; convex,[illegible]; capitate,[illegible].
lustre. shining,11; dull,[illegible]; waxy,3.
chromogenesis. white,11; grey,18; brownish,5.
potato discolored. +,13; -,21.

Gelatin colony.
form. round,34;
elevation. flat,16; raised,3; convex,6; umbilicated,[illegible],1.
edge. entire,31; lobed,1;serrated,1;auriculate,1.
internal structure. homogeneous,7; granular,[illegible]; grumose,10; marmorated,1.
chromogenesis. white,8; grey,13; brownish,6;brown,1; yellowish,1;bluish,5.
optical characters. transparent,2; translucent,[illegible]; paraffinous, 1; opalescent,4; nacreous,4; opaque,[illegible].

Agar colony.
form. round,34.
elevation. flat,13; raised,3; convex,17; umbilicate,1.
edge. entire,30; waved,1; lobed,1; serrated,1.ciliate,1.
internal structure. homogeneous,4; granular,[illegible]; grumose,[illegible]1.

Milk.
coagulation. positive,3[illegible]. negative,2.
liquefaction. positive,32; negative,2.
reaction. acid,34.

Micrococcus aureus. No. 12. 34 observations.

Agar slant.

form. filiform,[illegible]2; beaded,[illegible]; echinulate,[illegible];

elevation. flat,18; raised,12; convex,3; umbilicate,1.

lustre. shining,34.

chromogenesis. gray,13; brownish,1; orange,5; light orange,11; yellowish,4.

topography. smooth,34.

optical characters. translucent,6; o[illegible],31; sebaceous, 1; butyrous, 1; opaque, 24.

Gelatin stab.

form liquefaction. crateriform,7; saccate,1 ; infundibuliform, 2; napiform, 1; stratiform, [illegible];0,7.

line growth. filiform,[illegible]; 0,1[illegible].

surface growth. flat,1 no growth,33.

Broth.

pellicle. no pellicle,34.

turbidity. clears up on standing, 1; non-characteristic, 31; no turbidity, 2.

sediment. non-characteristic, 32; no sediment,2.

Potato.

form. filiform,2[illegible]; nodose, 1; beaded, [illegible]; echinulate,4;0,1.

elevation. flat,4; raised, 1[illegible]; convex,[illegible]; capitate,[illegible]; umbonate, 1; 0,1.

lustre. shining 27; dull, 5; waxy,1;0,1.

chromogenesis. brownish,[illegible]; orange,2; orange-brown,13; red-brown, 4; light orange, 3; yellowish,[illegible]; yellow-brown, 1; orange-yellow,1; 0,1.

potato discolored; +,17; 0,17.

Gelatin colony.

form. round,30; no growth,4.

elevation. flat,11; raised; 1[illegible]; convex,7; capitate,[illegible]; umbonate, 1; 0,1.

lustre. shining,23; dull,9, waxy,4; 0,1.

chromogenesis. brownish,2; orange,2; yellowish,[illegible]; orange-brown,13; red-brown, 4; light orange 3; yellow-brown, 1; orange-yellow, 1; 0,1.

Agar colony.

form. round,34.

elevation. flat,9; convex, 23; capitate,1; umbilicate,1.

edge. entire, 32; waved,2.

internal structure. homogeneous,7; granular,14; grumose,12; clouded,1;

Milk.
coagulated, 2[illegible]; not coagulated, 1[illegible].
liquefaction. +,13: -,21.
reaction. acid,3[illegible]; no change,[illegible].

Bacillus suipestifer, No. 13; 34 observations.

Agar slant.
form. filiform,18; echinulate,10; villous,5; spreading,1.
elevation. flat,24; effuse,1; raised,3; convex,5; umbonate,1;
lustre. shining,28; dull,5; waxy,1.
chromogenesis. white,3; gray,29; brown,1; brownish,1.
topography. smooth,33; contoured,1.
optical characters. transparent,2; translucent,4; opalescent, 27; opaque,1.
viscosity. +,[illegible]; 0,3[illegible].

Gelatin stab.
liquefaction. negative,34.
line growth. filiform,33; beaded,1.
surface growth. flat,30; 0,4.

Broth.
pellicle, +,2! negative,32.
turbidity. non-characteristic,34.
sediment, non-characteristic, 30; none,4.

Potato.
form. filiform, 30; echinulate,4.
elevation. flat,5; convex, 15; raised,11; capitate,2; effuse,1.
lustre. shining,29; dull,4; waxy,1.
chromogenesis. gray,4; brown, 17; yellowish,13.
potato discolored. +,[illegible]. 0,[illegible].

Gelatin colony.
form. round,24. no growth,10.
elevation. flat,9; raised,2; convex,13; 0,10.
edge. entire,22; lobed,1;.ciliate,1; 0,10.
internal structure. granular,18; homogeneous,6; 0,10.
chromogenesis. gray,15; brown,3; orange,1; yellow,4; blue, 1; 0,10.
optical characters. transparent,3; [illegible],4; opalescent, 10; nacreous,1; opaque,6; 0,10.

Agar colony.
form. round,32; amoeboid,1; 0,1.
elevation. flat,11; raised,[illegible]; convex,[illegible]0; 0,1.
edge. entire,29; waved,2; lobed,1; ciliate,1; 0,1.

Milk.

Milk.
coagulated. +,2; 0,31.
liquefaction. negative,34.
reaction. acid; [illegible]; alkaline,1; terminal alkalinity,24.

Bacillus alcaligenes. No. 14; 33 observations.

Agar slant.
form. filiform,8: echinate,14; villous,5; plumose,2;
spreading,4.
elevation. flat,18; effuse,3; raised,8; umbonate,8.
lustre. shining,[illegible]; dull,3; waxy,1.
chromogenesis. white,3; grey,[illegible]; brown,2; yellowish,2.
topography. smooth,[illegible]; contoured,4.
optical characters. translucent,25; transparent,4;
opaque,4;

Gelatin stab.
liquefaction. negative, 33.
line growth. filiform,33.
surface growth. flat,1[illegible]: effuse,[illegible]; convex,[illegible]; capitate,
4; 0,6.

Broth.
pellicle. +,4: no pellicle,29.
turbidity. slight,2; decided,31.
sediment, non-characteristic. 33.

Gelatin colony.
form. round,31; [illegible], 1;0,1.
elevation. flat,1[illegible]; convex,1[illegible]; umbilicate,1;0,1.
edge. entire,2[illegible] lobed,4; serrated,1; erose,1; 0,1.
internal structure. homogeneous,13; granular,12; hyal-
ine,1. areolate,1 grumose, 5; 0,1.
chromogenesis. gray,1[illegible]. brownish,5; yellowish,[illegible]; 0,1.
optical characters. translucent,[illegible]; opalescent,1[illegible];
ceraceous,1; opaque,8; 0,3.

Potato.
form. filiform,29. echinate,4;
elevation. flat,[illegible]; raised,1[illegible]; convex,1[illegible]; [illegible],[illegible];
lustre. shining,33.
chromogenesis. gray,1; brownish,1[illegible]; reddish,[illegible]; orange
[illegible]; yellow, 10.
potaot discolored. +,31;0,2.

Agar colony.
form. round,31; [illegible]iform,2.
elevation. flat,6; convex,26; umbilicate,1.
edge. entire,25; waved,5; lobed,2; serrated,[illegible]; erose,1.

internal structure. homogeneous, [illegible]; granular, [illegible]; areolate, 2; grumose, [illegible]; moruloid, 1.

Milk.

coagulated, negative, [illegible].

liquefaction. negative, [illegible].

reaction. alkaline, 32; terminal alkalinity, 1.

Bacillus dysenteriae, (Shiga) No. 15; 34 observations.

Agar slant.

form. filiform, 17; echinate, [illegible]; villous, [illegible]; plumose, [illegible]; spreading, 1.

elevation. flat, 2[illegible]; effuse, 2; raised, [illegible]; convex, 5. umbilicate, 1.

lustre. shining, 31; waxy, [illegible].

chromogenesis. gray, 27; white, 2; brownish, [illegible].

topography. smooth, 34.

optical characters. transparent, 1; translucent, 3; opalescent, 30.

Gelatin stab.

liquefaction. negative, 34.

line growth. filiform, 3[illegible]; beaded, 1; plumose, 1.

surface growth. flat, 28; raised, 3; convex, [illegible].

Broth.

pellicle. no pellicle, 34.

turbidity. slight, 17; decided, 17.

sediment. non-characteristic, 34.

Potato.

form. filiform, 2[illegible]; echinate, [illegible]; spreading, 1.

elevation. raised, 1[illegible]; convex, 13; capitate, 5.

lustre. shining, 2[illegible]; dull, 5; wax[illegible], 3.

chromogenesis. gray, 5; brownish, 10; bluish, 1; yellow, 1[illegible].

potato discolored. +, 18: -, 16.

Gelatin colony.

form. round, [illegible]; no growth, 14.

elevation. flat, 7; raised, [illegible]; convex, [illegible]; 0, 14.

lustre. shining, 1[illegible]; dull, 2; 0, 14.

internal structure. homogeneous, 7; granular, 13; grumose, 1; 0, 14.

chromogenesis. white, 1; gray, 1[illegible]; yellowish, 3; 0, 14.

Agar colony.

form. round, 3[illegible]; filiform, [illegible].

elevation. flat, 13; effuse, [illegible]; raised, 3; convex, 1[illegible].

edge. entire, 2[illegible]; wavy, 3; serrated, 1; erose, 1; ciliate, 1.

internal structure. homogeneous, [illegible]; granular, 1[illegible]; grumose, 1[illegible].

Milk.

coagulated, negative, 34.

liquefaction. negative, 34.

reaction. acid, [illegible]; alkaline, 1. terminal alkalinity, 1[illegible].

Bacillus dysenteriae, (Harris) No. 1[illegible]; 34 observations.

Agar plant.

form. filiform, 2[illegible]; echinate, [illegible]; villous, [illegible]; plumose, 1.

elevation. flat, 30. effuse, [illegible]; raised, [illegible].

lustre. shining, 30; dull, 3. waxy, 1.

chromogenesis. white, 2; grey, 27; brownish, [illegible].

topography. smooth, [illegible].

optical characters. transparent, 2; translucent, [illegible];
opalescent, 25; butyrous, 1; opaque, 1.

viscosity. +1. -, 33.

Gelatin stab.

liquefaction. negative, 34.

line growth. filiform, 31; beaded, 1; echinate, 1; plumose, 1.

surface growth. flat, 1; raised, 2; convex, [illegible]; [illegible], [illegible].

Broth.

pellicle. +, 2; -, 32.

turbidity. slight, 17; decided, 17.

sediment. non-characteristic, 34.

Potato.

form. filiform, [illegible]; echinate, [illegible].

elevation. flat, [illegible]; raised, [illegible]; convex, 14; capitate, 3;
umbilicate, [illegible].

lustre. shining, [illegible]7; dull, [illegible]; waxy, [illegible].

chromogenesis. white, [illegible]; grey, [illegible]; brownish, 1; yellow-
ish, 3; 0, [illegible].

potato discolored. +, 23. 0, 11.

Gelatin colony.

form. round, 34.

elevation. flat, 1[illegible]; convex, 1[illegible]; capitate, 1; 0, [illegible].

lustre. shining, 29; dull, 2; waxy, 1; 0, 2.

internal structure. homogeneous, [illegible]; granular, [illegible]; gru-
mose, 3; moruloid, 1; 0, 2.

chromogenesis. white, 5. grey, [illegible]; brownish, 1; yellowish, [illegible].

Agar colony.

form. round, [illegible]. fusiform, [illegible].

elevation. flat, 11; convex, 21; umbilicate, 2.

edge. entire, 2[illegible]; serrated, [illegible]; ciliate, [illegible]; erose, [illegible].

internal structure. homogeneous, 7; granular, 13;
grumose, 13; [illegible]rose, 1.

Milk coagulated. negative, 34.

[illegible]
[illegible]

Bacillus enteritidis. No. 17; 34 observations.

Agar slant.

form. filiform,23; nodose, 1; echinate,9; villous,1.
elevation. flat,26; raised,3; convex,4; umbonate,1.
lustre. shining, 30; dull, 1; waxy,3.
chromogenesis. gray,29; white,1; brownish,4.
topography. smooth,30; alveolate,1; punctate,3.
optical characters. translucent, 1; opalescent, 27; nacreous, 1; opaque, 5.

Gelatin stab.

form of liquefaction, crateriform,1; no liquefaction,33.
line of growth. filiform,29; beaded,2; plumose,2;0,1.
surface growth. flat,10; umbilicate,1; umbonate, 2; convex, 2; 0,19.

Broth.

pellicle, +,2;-,32.
turbidity. clears up on standing,1; slight,23; decided,10.
sediment. non-characteristic.34.

Potato.

form. filiform,26; echinate, 4; spreading,4.
elevation. flat, 3; raised,18; convex, 12; umbonate,1.
lustre. shining, 25; dull,2; waxy,7.
chromogenesis. gray, 4; brownish, 13; reddish, 14; yellowish, 1[illegible];
potato discolored. +,28; -,6.

Gelatin colony.

form. round,28; 0,6.
elevation. flat, 3; raised, 1[illegible]; convex, 12; umbonate,1.
edge. entire,30; wavy, 1; auriculate, 1;0,2.
internal structure. homogeneous, 7; granular, 18; grumose, 3; 0,6.
chromogenesis. white,2; gray,14; brownish, 4; yellowish, 4; blue-brown, 4. 0,6.
optical characters. transparent, 2; translucent, 1; paraffinous,1; opalescent,14; nacreous, 2; opaque, 7; 0,6.

Agar colony.

form. round,30; filiform,2; conglomerate,1; 0,1.
elevation. flat,6; raised,2; convex, 24; umbilicate,1; 0,1.

edge. entire, 30; serrated, 1; erose, 2; 0,1.
internal structure. homogeneous, 5; granular, 21; grumose, 7; 0,1.

Milk.
coagulated; negative, 34.
liquefaction. negative, 34.
reaction. acid, [illegible]; alkaline, 0; terminal alkalinity, [illegible].

Bacillus paracoli. No. 18; 34 observations.

Agar slant.
form. filiform, [illegible]; beaded, 5; echinate, 1; plumose, [illegible]; arborescent, 1.
elevation. flat, 32; effuse, 1; raised, 1.
lustre. shining, 30; dull, 1; waxy, [illegible].
chromogenesis. white, 1; gray, 26; brownish, 7.
topography. smooth, 34.
optical characters. transparent, 1; translucent, 4; opalescent, 28; opaque, 1.

Gelatin stab.
liquefaction. negative, 34.
line growth. filiform, 33; echinate, 1.
surface growth. flat, [illegible]; raised, 1; convex, 5; effuse, 1. umbilicate, 1; 0,10.

Broth.
pellicle. +,4 -,30.
turbidity. slight, 12; decided, 22.
sediment. non-characteristic, 34.

Potato.
form. filiform, [illegible]; echinate, 5; spreading, 3;
elevation. flat, [illegible]; effuse, 1; raised, [illegible].
lustre. shining, 17; dull. 1; waxy, 16.
chromogenesis. gray, 3; brownish, 22; yellowish, 5; bluish, 4.
potato discolored. +,31. -,3.

Gelatin colony.
form. round, 26; conglomerate, 1; 0,7.
elevation. flat, 10; raised, 4; crown, [illegible]; umbilicate, 1.
edge. entire, 24; lobed, 2; erose, 1; 0,7.
internal structure. homogeneous, 6; granular, 20; grumose, 1; 0.7.
optical characters. transparent, 1; translucent, [illegible]; paraffinous, 3; opalescent, [illegible]; sebaceous, 1. opaque, 5; 0,7.

chromogenesis. white,4; gray,[illegible]; brownish,[illegible]; 0,7.

Agar colony.

form. round,33. fusiform,1.

elevation; flat, 34.

edge. entire, 30; serrated, 1; erose,3.

internal structure. homogeneous, 3; granular, 21; grumose, 10.

Milk.

coagulated. negative,34.

liquefaction. negative,34.

reaction. alkaline,2; acid,3; terminal alkalinity,[illegible].

Bacillus of Friedlaender. No. 1[illegible]. 34 observations.

Agar slant.

form. filiform,16; beaded,1; villous,1; echinate,15; plumose, 1.

elevation. flat,5; raised, 17; convex,[illegible]; umbonate,[illegible]; umbilicate,1. capitate,4.

lustre. shining,34.

chromogenesis. white,[illegible]; gray, 26; brownish,7.

optical characters. transparent, 1; translucent,1; opalescent, 20; opaque,1[illegible].

viscosity. +,30; 0,4.

Gelatin stab.

liquefaction. negative,34.

line growth. filiform,[illegible]; beaded,2; nodose,[illegible].

surface growth. flat,[illegible]; raised,[illegible]; convex,21; 0,1.

Broth.

pellicle. 0,34.

turbidity, slight,21; decided, 13.

sediment. non-characteristic, 34.

Potato.

form. filiform,23; echinate,7; spreading,[illegible].

elevation. raised,13; convex,7; capitate,11; umbilicate, 3.

lustre. shining, 30; dull,1; waxy,3.

chromogenesis. gray,[illegible]; brownish, [illegible]; yellowish,[illegible].

potato discolored. +,34.

Gelatin colony.

form. round,29; rosulate, 1; 0,4.

elevation. flat,9; raised,1; convex,1[illegible]; umbilicate,1.

edge. entire, [illegible]9; lobed, 1; 0,4.

internal structure. homogeneous,[illegible]; granular,17; grumose, 3; [illegible], 1; 0,4.

chromogenesis. white,6; gray,13; brownish,2; reddish,1. yellowish, 8; 0,4.

optical characters. transparent, 3; vitreous, 1; oleaginous, 1; translucent, 3; [illegible], 3; opalescent 3; opaque, 13; 0,4.

Agar colony.

form. round, 32; rosulate,1; 0,1.

elevation. flat,2; raised,3; convex, 27; umbilicate,1.

edge. entire,30; erose,2; lobed,1; 0,1.

internal structure. homogeneous, 1; granular, 25; grumose, 5; 0,1.

Milk.

coagulated, +,2; no coagulation,32.

liquefaction. negative,34.

reaction. acid,33; terminal alkalinity,1.

Bacterium aerogenes. No. 10. 34 observations.

Agar slant.

form. filiform,23; echinulate,8; villous,2; spreading,1.

elevation. flat,8; effuse,1; raised,11; convex,11; [illegible],2; umbilicate,1.

lustre. shining,32; dull,2.

chromogenesis. white,4; gray,28; brownish,2.

optical characters. translucent, 2; opalescent, 20, opaque,12;

viscosity. +,14; -,20.

Gelatin stab.

form of liquefaction. 34 negative.

line of growth. filiform,25; beaded,8; [illegible],1.

surface growth. flat,15; raised,8; convex,4; 0,3.

Broth.

pellicle, 0,34.

clouding. slight,[illegible]; decided, 34.

sediment. non-characteristic,34.

Potato.

form. filiform, 23; echinulate, 5; spreading,6.

elevation. flat,4; effuse,1; raised,14; convex,9; capitate, 6.

lustre. shining, 31; dull,2; waxy,1.

chromogenesis. white,1; gray, 4; brownish, 16; yellowish, 13.

potato discolored. +,27; -,7.

Gelatin colony

form. round, 25; amoeboid, 2; 0,7.

elevation. flat,4; raised, 8; convex, 15; 0,7.

edge. entire,2[illegible]; lobed,[illegible]; waved,1; erose,1; 0,7.
internal structure. homogeneous, 8; grumose,17; granular, 2; 0,7.
chromogenesis. white,5; gray,1[illegible]; brownish, 4; reddish, 1; purple,1; 0,7.
optical characters. transparent, 1; translucent, 3; paraffinous, 3; opalescent, 9; nacreous,1; sebaceous, 2; opaque,8; 0,7.

Agar colony.
form, round, 32; amoeboid, 1; 0,1.
elevation. flat,1; effuse,1 raised,4; convex,27; 0,1.
edge. entire,32; ciliate, 1; 0,1.
internal structure. homogeneous, 7; granular, 1[illegible]; grumose, 5; 0,1.

Milk.
coagulated, +.33; -,1.
liquefied. +,11; -,23.
gas. +, 5; -,29.
reaction.acid,34.

Bacillus mirabilis. No. 21; 34 observations.

Agar slant.
form. filiform,3; echinate,[illegible]; villous,4; spreading,[illegible].
elevation. flat,21: effuse,7; raised,4; convex,2.
lustre. shining,34.
chromogenesis. white,[illegible]; gray,23; brownish,[illegible].
topography. smooth,33; contoured,1.
optical character. transparent,1; resinous,1; translucent,[illegible]; opalescent,12; sebaceous,4; [illegible]rous, 1; opaque,6.
viscosity. +,3; -,31.

Gelatin stab.
form liquefaction. saccate,[illegible]; infundibuliformii; napiform,4; stratiform,13; no liquefaction, 2.
line growth. filiform,22; no growth,12.
surface growth. convex,4; no growth,30.

Broth.
pellicle. +,4; -,30.
turbidity. slight,1; decided,33.
sediment. non-characteristic,34.

Potato.
form. filiform,2[illegible]; echinate,8; spreading,3.
elevation. flat,8; raised,8; convex,1[illegible]; capitate,1. umbilicate,1.

lustre. shining,28; dull,5; waxy,1.
chromogenesis. gray,4; brownish,5; brown-red,4; yellowish, [illegible]; yellow-orange,6.
potato discolored. +,31; -,3.
gas. +,[illegible]; -,[illegible].

Gelatin colony.
form. round,13; rhizoid,1; no growth,20.
elevation. flat,11; effuse,2; raised,1; -,20.
edge. entire, 7; wavy,2; lobed,1; ciliate,3; erose,1.
internal structure. homogeneous,1; granular,4; areolate, 2; grumose, 5; 0,20.
chromogenesis. white,3; gray,7; brownish,4; 0,20.
optical characters. translucent, 6; opalescent,4; opaque,4; 0,20.

Agar colony.
form. round,23; amoeboid,2; myceloid,2; 0,2.
elevation. flat,11; raised,9; convex,2;capitate,10. 0,2.
edge. entire, [illegible]; auriculate,2; ciliate,1; erose,7. 0,[illegible].
internal structure. homogeneous,3; granular,[illegible]; grumose, 5; 0,[illegible].

Milk.
coagulated. +,24; -,10.
liquefaction. +,17: -,17.
reaction. acid,31; alkaline,1. amphoteric,2.

Bacillus vulgaris. No. 23. 34 observations.

Agar slant.
form. filiform,4; echinulate,2; arborescent,1; spreading, 27.
elevation. flat,[illegible]; raised,5; convex,1.
lustre. shining,33; dull,1. waxy,1.
chromogenesis. white,3; gray,12; brownish,6; yellowish,[illegible].
topography. smooth,27; contoured,4; papillate,[illegible]; rugose,1.
optical characters. transparent,1; translucent,[illegible]; opalescent,22; iridescent,1; fluorescent,1; opaque,[illegible].
viscosity. +,8; -,[illegible].

Gelatin stab.
liquefaction. crateriform,6; saccate,3; infundibuliform, 3; napiform,1; stratiform,14; -,4.
line growth. filiform,13; [illegible],2; beaded,1; villous,2; no growth,11.
surface growth. convex,4; 0,3[illegible].

Broth.

pellicle. no pellicle,34.

turbidity.slight,[illegible]; [illegible],[illegible].

sediment.non-characteristic,3[illegible].

Potato.

form.filiform,[illegible]; echinulate,1; arborescent,1; spreading,[illegible].

elevation.flat,[illegible]; effuse, 1[illegible]; convex,3; capitate,1.

lustre.shining, [illegible]3. dull.4; waxy,[illegible]; iridescent,[illegible].

chromogenesis.brownish,1[illegible]; yellowish,7; yellow-brown, 7; orange,4.

potato discolored. +,3[illegible]. -,[illegible].

gas.+,[illegible]; -,3[illegible].

Gelatin colony.

form.round, 1[illegible]; rhizoid, [illegible]; -,13.

elevation.flat,[illegible]; effuse,[illegible]; raised,[illegible]; convex,[illegible]; 0,1[illegible].

edge.entire, 11; undulate,1; lobed, 1; serrate,4; auriculate, 1; erose,3; 0,13.

internal structure.homogeneous,[illegible]; granular,1[illegible]; grumose, 1; 0,13.

chromogenesis.white,[illegible]; grey,1[illegible]; brownish,[illegible]; 0,13.

optical characters.[illegible],1; opalescent,9; opaque, 11; 0,13.

Agar colony.

form.round,23; conglomerate,[illegible]; amoeboid,4; [illegible],2; filamentous,[illegible]; 0,1.

elevation.flat,5; raised, [illegible]; capitate,6; 0,1.

edge.entire,5; lobed,[illegible]; auriculate,6; 0,1.

internal structure. homogeneous,[illegible]; granular, 2; grumose, 6; 0,1.

Milk.

coagulated, +,1[illegible]; -,2[illegible].

liquefaction.+,14- -,[illegible].

reaction. acid,13: alkaline,[illegible]: terminal alkalinity,11.

Bacillus acidi lactici. No. [illegible]. 34 observations.

Agar slant.

form.filiform,1[illegible]; echinulate,[illegible]; villous,[illegible]; arborescent, 3; spreading,2.

elevation.flat, 1[illegible]; effuse, 2; raised, [illegible]; convex,[illegible]; umbonate,1; umbilicate,1.

lustre;shining,[illegible]3; waxy,1.

chromogenesis;white,[illegible]; grey,[illegible]3; brownish,5.

topography.smooth, [illegible]1; contoured,3.

optical characters.translucent,[illegible];opalescent, [illegible]7; opaque,[illegible].

viscosity. [illegible],14; -,2[illegible].
Gelatin stab.
form liquefaction. [illegible],1; no liquefaction,[illegible].
line growth. filiform,31; [illegible],1; [illegible],[illegible].
surface growth. flat,28; 0, 5.
Broth.
pellicle, +,1; 0, 33.
turbidity, slight,4; decided,30.
sediment. non-characteristic,28; no sediment,[illegible].
Potato.
form. filiform,[illegible]; [illegible],4; spreading,1[illegible].
elevation. flat,5; effuse,1; raised,13; convex,1[illegible];
capitate,1.
lustre. shining, 20; dull, [illegible]; waxy, [illegible].
chromogenesis. white,1; gray,9; brownish,1[illegible]; [illegible],[illegible];
orange,[illegible]; red-orange,1; yellowish, [illegible].
potato discolored. +,30. -, 4.
Gelatin colony.
form. round,[illegible]; amoeboid,1; 0, 1[illegible].
elevation. flat,[illegible]; raised,[illegible]; convex,13; 0,1[illegible].
edge. entire, 18; waved,4; lobed,1; [illegible],1[illegible].
internal structure. homogeneous,[illegible]; granular,14; gru-
mose, 7; 0, 1[illegible].
chromogenesis. white, [illegible]; gray, 14; brownish, 3; yellow,
1; 0, 10.
optical characters. translucent,[illegible]. paraffinous,1; opal-
escent, 1[illegible]; [illegible], [illegible]; nacreous, 1; opaque,[illegible].
0, 10.
Agar colony.
form. round, 2[illegible]; fusiform, [illegible]; amoeboid,1; rhyzoid,1.
no growth, [illegible].
elevation. flat, [illegible]; effuse, 1. raised, 10; convex, 1[illegible];
0, [illegible].
edge. entire, [illegible]; ciliate, 1; erose, 2; [illegible], [illegible].
internal structure. homogeneous, 3; granular, 15; gru-
mose, 14; 0, [illegible].
Milk.
coagulated. +,[illegible]8; -, [illegible].
liquefaction. [illegible],3; -, 31.
gas. +, [illegible]; -, [illegible].
reaction. acid,33. amphoteric, 1.

Bacillus [illegible]. [illegible]o. 2[illegible]. 33 observations.

Agar slant.
form. filiform,1[illegible]; [illegible],1; nodose, 1; echinulate, [illegible];
villous,4; [illegible], [illegible]; arborescent,[illegible].

elevation. flat, 13; effuse, [illegible]; raised, [illegible]; convex, 1.
lustre. shining, 28; dull, 4; waxy, 1.
chromogenesis. white, [illegible]; gray, [illegible]; brownish, 1.
topography. smooth, [illegible]; rugose, 1; contoured, [illegible].
optical characters. transparent, 5; vitreous, 1; translucent, [illegible]; [illegible], 3; opalescent, 11; opaque, 3.
viscosity. negative, 33.

Gelatin stab.
liquefaction. infundibuliform, 1; no liquefaction, [illegible].
line growth. filiform, [illegible]; no growth, 11.
surface growth. flat, 3; no growth, 30.

Broth.
pellicle. no pellicle, 33.
turbidity. slight, 25; medium, 7; no turbidity, 1.
sediment. non-characteristic, [illegible]. no sediment, 1.

Potato.
form. filiform, 20; beaded, 4; 0, 3;
elevation. flat, 7; effuse, 4 raised, 14; convex, [illegible]; 0, [illegible].
lustre. shining, 14; dull, [illegible]; waxy, 1; 0, 3.
chromogenesis. white, 5; gray, 7; brownish, 3; yellowish, [illegible]; 0, 3.
potato discolored. +, 19; -, 14.

Gelatin colony. form, round, [illegible]; conglomerate, 2; mycelioid, 2; 0, 20.
elevation. flat, [illegible]; effuse, 1; raised, 3; convex, 1; 0, 20.
edge. entire, 7; waved, [illegible]; lobed, [illegible]; ciliate, 2. 0, 20.
internal structure. granular, 10; [illegible], 2; marmorated, 10; 0, 20.
chromogenesis. white, 1; gray, 3; yellowish, 4; 0, [illegible].
optical characters. translucent, 4; transparent, 1; [illegible], 3; opalescent, [illegible]; opaque, 3., 0, [illegible].

Agar colony.
form. round, [illegible]; amoeboid, [illegible]; filamentous, 3; rosulate, 2; 0, [illegible].
elevation. flat, 5; effuse, 4; raised, 7; convex, [illegible]; umbilicate, 2; 0, 2.
edge. entire, 17; waved, 2; lobed, 1; serrated, 4; erose, 7; 0, 2.
internal structure. granular, [illegible]; grumose, 3; reticulate, 1; [illegible], [illegible]; marmorated, 1; 0, 2.

Milk.
coagulated. -, [illegible]. +, 7.

[illegible]. +,5; -, 30.
reaction. acid, 15; alkaline, [illegible]; amphoteric, [illegible].

Bacillus pyocyaneus. No. [illegible]; 34 observations.

Agar slant.
form. filiform, 20; beaded, 4; echinulate, 2; spreading, 8.
elevation. flat, 21; raised, 10; effuse, 2. [illegible], 1.
lustre. shining, 9; dull. 0. iridescent. 25.
chromogenesis. white, 2; gray, 10. yellow-green, 1; green, [illegible]; greenish, 7; green-red, 1; dark-green, 1; blue, 1; dark blue, 1.
topography. smooth, 24; bullate, 3; rugose, 7.
optical characters. translucent, 11; opalescent, [illegible]; opaque, [illegible].
viscosity. +,13; -, 22.

Gelatin stab.
liquefaction. crateriform, 15; saccate, 6; napiform, [illegible]; no liquefaction, 6.
line of growth. form, filiform, 25; 0, [illegible].
surface growth. flat, [illegible]; effuse, 2; [illegible], 1; 0,6.

Broth.
pellicle. +,23- -,11.
turbidity. clears up on standing, 1; slight, 1; decided, 3[illegible].
sediment. non-characteristic, 34.

Potato.
form. filiform. 27; nodose, 2; echinulate, 1; spreading, 5.
elevation. flat, 5; effuse, 1; raised, 10; convex, 15 capitate, 1.
lustre. shining, 19; dull, 5; waxy, 3; iridescent, 7.
chromogenesis. brownish, 5; brown, 4; brown-yellow, 4 pink, 12; red-brown, 3; greenish, 1.
potato discolored, +,32. -, [illegible].

Gelatin colony.
form. round, 30. cochleate, 1; filamentous, 1; 0, 2.
elevation. flat, 5 raised, 3; convex, 20. [illegible] = 0.
edge. entire, 19; waved, 11; ciliate, [illegible]; 0, [illegible].
internal structure. homogeneous, 1; granular, 20; grumose, [illegible]; moruloid, 1; 0. [illegible], 3.
chromogenesis. white, 2; gray, 4; brownish, 4; yellowish, 1; green-brown, 1 ; 0, [illegible].
optical characters. transparent, 3; translucent, 9, opalescent, 3; [illegible], 5; [illegible], 1; opaque, 11; 0, [illegible].

Agar colony.
form. round, 3[illegible]; amoeboid, 1; rosulate, 1.

elevation. flat, 14; effuse, [illegible]; raised, 1[illegible]; convex, [illegible];
capitate, [illegible]; umbilicate, 2.

edge. entire, 2[illegible]; waved, 5; lobed, [illegible]; auriculate, 1;
erose, [illegible].

internal structure. homogeneous, 1; granular, 1[illegible]; grumose, 15; gyrose, 1.

Milk.

coagulated, +,28; -,6.

liquefaction. +,30. -, 4.

reaction. acid, 20. alkaline, 10. amphoteric, 4.

Bacillus subtilis. No. 26. 34 observations.

Agar slant.

form. filiform, [illegible]; plumose, 4; arborescent, [illegible]; spreading, 2.

elevation. flat, 1[illegible]; effuse, 5. raised, 1[illegible].

lustre. shining, [illegible]; dull, 4; waxy, 2[illegible].

chromogenesis. white, [illegible]; gray, 29; brownish, 3.

topography. smooth 2[illegible]; alveolate, 1; punctiform 2,
rugose, 2.

optical characters. transparent, 1; paraffinous, 1;
opalescent, 1; opaque, 31.

viscosity. +,[illegible]. -,31.

Gelatin stab.

liquefaction. crateriform, [illegible]; infundibuliform, [illegible];
stratiform, 21.

line growth. filiform, 21; beaded, [illegible]; villous, 1. 0, 10.

surface growth. flat, 5; 0, 2[illegible].

Broth.

pellicle. +,16; -,18.

turbidity. clears up on standing, [illegible]; slight, 13. decided, 7; no sediment, 7.

Potato.

form. filiform, 1[illegible]. beaded, 1; echinulate, [illegible]; spreading, 11.

elevation. flat, 5; effuse, 1; raised, 24; convex, [illegible];
capitate, 2.

lustre. shining, 6; dull, 23; waxy, [illegible].

chromogenesis. white, 2; gray, 6; brownish, 10. reddish,
2; yellowish, 11; greenish, 3.

potato discolored. +,30. -,4.

gas. +,2. -, 32.

Gelatin colony.

form. round, 13; filamentous, 10, 0, 11.

elevation. flat, 14; effuse, 3; raised, 5; convex, 1.
0, 11.

edge. entire, 8; waved, 3; serrated, [illegible]; ciliate, 7;
erose, 3.
internal structure. homogeneous, [illegible]; granular, 4; grumose, 4; moruloid, 5; gyrose, [illegible]; 0, 11.
chromogenesis. white, 3. gray, 18; yellowish, [illegible]. 0, 11.
optical characters. translucent, 2; opalescent, 2; opaque, 19; 0, 11.

Agar colony.
form. round, 4; myceloid, 1; filamentous, [illegible]; rhizoid, 3; rosulate, 4; 0, 1.
elevation. flat, 15; effuse, [illegible]; raised, [illegible]; convex, 2; umbilicate, 5; 0, 1.
edge. entire, [illegible]; ciliate, 25; erose, 1; 0, 1.
internal structure. granular, 6; moruloid, [illegible]; clouded, 1; gyrose, 1[illegible]; marmorated, 1; grumose, 3, 0, [illegible].

Milk.
coagulated. +,13; -, 21.
liquefaction. +,32. -,2.
reaction. acid, 31. alkaline, 2; amphoteric, 1.

Spirillum metschnikovi. No. 27. 34 observations.

Agar slant.
form. filiform, 27; echinate, [illegible]; plumose, 1; spreading, 2.
elevation. flat, 1[illegible]; effuse, 1; raised, 16; convex, 5
lustre. shining, 30; dull, 1; waxy, 3.
chromogenesis. white, [illegible]; gray, 11; brownish, 16; brown-yellow, 2; red-brown, 1; yellowish, 2.
topography. smooth, 33; rugose, 1.
optical characters. translucent, 3; opalescent, [illegible]1; sebaceous, 1; opaque, [illegible];
viscosity. +,3. -,31.

Gelatin stab.
form liquefaction. crateriform, 2; saccate, 16; infundibuliform, 13; stratiform, 3.
line growth. filiform, 7; nodose, [illegible]; beaded, 2; 0, 2[illegible].
surface growth. flat, 20; effuse, 2; raised, 4; convex, [illegible].

Broth.
pellicle. 34.
turbidity. slight, 10; decided, 24.
sediment. non-characteristic, 34.

Potato.
form. filiform, 26; echinate, 4; spreading, 2; 0, 2.
elevation. flat, [illegible]; raised, 1[illegible]; convex, 7; 0, [illegible].
lustre. shining, 27; dull, 4; iridescent, 1; 0, 2.

chromogenesis. gray, 1; brownish, [illegible]; reddish, [illegible]; yellowish, 3; 0, [illegible].
potato discolored. +, [illegible]; -, [illegible].

Gelatin colony.
form. round, 17; 0, 17.
elevation. flat, 14; raised, 1; umbilicate, 1; 0, 18.
edge. entire, 12; waved, 1; serrated, 2; erose, 2; 0, 17.
internal structure. homogeneous, 2; granular, [illegible]; grumose, 7; 0, 17.
chromogenesis. white, 2; gray, 3; brownish, 6; yellowish. 1; 0, 17.
optical characters. translucent, 2; opalescent, [illegible]; butyrous, 2; opaque, 6,. 0, 17.

Agar colony.
form. round, [illegible]; 0, 5.
elevation. flat, 2; raised, 5; convex, 23; 0, 5.
edge. entire, [illegible]; waved, 4; auriculate, 2; 0, 5.
internal structure. gray, 11; grumose, 18; 0, 5.

Milk.
coagulated. +, 19; -, 15.
liquefaction. +, 18; -, 16.
reaction. acid, 24; alkaline, 7; amphoteric, 3.

Bacillus anthracis. [illegible]. 28; 34 observations.

Agar slant.
form. filiform, 9; villous, 11; echinate, [illegible]; plumose, 1; spreading, 10.
elevation. flat, 11; effuse, 4; raised, 18; convex, 1.
lustre. shining, 19; dull, 8; waxy, 7.
chromogenesis. white, [illegible]; gray, [illegible]; brownish, [illegible].
topography. smooth, [illegible]; punctate, 4; rugose, 2; contoured, [illegible].
optical characters. opalescent, 1; opaque, [illegible].
viscosity. +, [illegible]; -, [illegible].

Gelatin stab.
liquefaction. crateriform, 3; saccate, 6; infundibuliform, 11; napiform, 6; stratiform, 5; 0, 3.
line growth. filiform, [illegible]; beaded, 2; villous, 3 plumose, 2; 0, 6. arborescent, 7.
surface growth. pellicle, [illegible]; no pellicle, 24.

Broth.
pellicle, 15 no pellicle, 19.
turbidity. clears up on standing, 10; slight, 3; absent, 21.
sediment. non-characteristic, 34.

Potato.
form. filiform, 12; villous, 1; spreading, 21.

elevation. flat, 18; effuse, 5; raised, 11; convex, 2.
lustre. shining, 1; dull, 30; waxy, 3.
chromogenesis. white, 7; gray, 5; brownish, 18; brown-red, 1; yellowish, 2.

potato discolored. +, 27; -, 7.

Gelatin colony.
form. round, 7; myceloid, 1; filamentous, 17; 0, 9.
elevation. flat, 13; effuse, 2; raised, 2; 0, 17.
edge. entire, 3; wavy, 1; serrated, 1; ciliate, 12; 0, 17.
internal structure. hyaline, 2; granular, 8; grumose, 8; 0, 16.
chromogenesis. white, 4; gray, 11; brownish, 2; 0, 17.
optical characters. translucent, 2; paraffinoid, 1; opalescent, 1; opaque, 13; 0, 17.

Agar colony.
form. round, 8; myceloid, 4; filamentous, 20. 0, 2.
elevation. flat, 11; effuse, 10; raised, 3; convex, 4; umbilicate, 4; 0, 2.
edge. entire, 3; lobed, 1; auriculate, 1; ciliate, 26; erose, 1; 0, 2.
internal structure. granular, 11; grumose, 4; gyrose, 16; moruloid, 1; 0, 2.

Milk.
coagulated. +, 27; -, 7.
liquefaction. +, 16. -, 18.
reaction. acid, 23; alkaline, 7; amphoteric, 4.

Spirillum tyrogenum. No. 29. 34 observations.

Agar slant.
form. filiform, 14; echinate, 7; arborescent, 1; spreading, 12.
elevation. flat, 12; effuse, 1; raised, 10; convex, 11.
lustre. shining, 33; dull, 1.
chromogenesis. white, 2; gray, 17; brown, 7; yellowish, 5; greenish, 3.
topography. smooth, 34.
optical characters. oleaginous, 1; opalescent, 19; sebaceous, 3; butyrous, 1; opaque, 10.

Gelatin stab.
liquefaction. saccate, 15; infundibuliform, 15; crateriform, 4.
line of growth. filiform, 5; nodose, 2; 0, 27.
surface growth. flat, 1; raised, 2; 0, 31.

Broth.

pellicle. +,18; 0, 16.

turbidity. clears up on standing, 3; slight, 5; [illegible] 29.

sediment. non-characteristic, 34.

Potato.

form. filiform, 30. echinulate, 4.

elevation. flat, 19; effuse, 2; raised, 7; convex, 6.

lustre. shining, 30. waxy, 4.

chromogenesis. white, 3; gray, 4; brown, 14; yellowish, 19; greenish, 1.

potato discolored. +, [illegible]; 0, 23.

Gelatin colony.

form. round, 16; 0, 18.

elevation. flat, 5; raised, 2; convex, 8; 0, 19.

edge. entire, 14; erose, 2; 0, 18.

internal structure. homogeneous, 1; granular, 14; grumose, 1; 0, 18.

chromogenesis. gray, 15; yellowish, 1; 0, 18.

optical characters. resinous, 1; paraffinous, [illegible]; opalescent, [illegible]; [illegible], 2; translucent, 4; opaque, 2.

Agar colony.

form. round, 31; fusiform, 1; amoeboid, 1; rhizoid, 1; 0, 18.

elevation. flat, 9; raised, 3; convex, 17.

edge. entire, 2[illegible]; waved, 7; lobed, 1; serrated, 3; auriculate, 1;

internal structure. granular, 16; grumose, 15.

Milk.

coagulated. +, 15; 0, 16.

liquefaction. +,20; -, 14.

reaction. acid, 32; amphoteric, 2.

Bacillus cuniculicida. No. 30. 33 observations.

Agar slant.

form. filiform, 18; beaded, 1; villous, 1; echinulate, 9. spreading, 4.

elevation. flat, 27; effuse, 4; raised, 2.

lustre. shining, 31; dull, 2.

chromogenesis. white, 3; gray, 29; brownish, 1.

topography. smooth, 31; alveolate, 2.

optical characters. transparent, 10; vitreous, 2; translucent, 7; opalescent, 13; opaque, 1.

<u>Gelatin stab</u>.
  <u>liquefaction</u>. negative,33.
  <u>line growth</u>.filamentous,19; plumose, 1; 0, 13.
  <u>surface growth</u>.flat,2; 0, 31.
<u>Broth</u>.
  <u>pellicle</u>. +, 2; 0, 31.
  <u>turbidity</u>. clears up on standing, [illegible]; slight, 21; decided, [illegible].
  <u>sediment</u>. non-characteristic,30. no sediment,3.
<u>Potato</u>.
  <u>form</u>. filiform, 5; no growth, 28.
  <u>elevation</u>.flat, 5; effuse, 2; 0, 26.
  <u>lustre</u>; glistening,5; 0, 28.
  <u>chromogenesis</u>.white,1; grey,3; yellowish,1; 0,28.
  <u>potato discolored</u>. +,1; 0, 32.
<u>Gelatin colony</u>.
  <u>form</u>.round, 7; no growth,26.
  <u>elevation</u>.flat,1; raised,3;convex, 2; umbonate,1; 0,26.
  <u>edge</u>.entire. 6; lobed,1; 0, 26.
  <u>internal structure</u>. homogeneous,2; granular,5; 0,26.
  <u>chromogenesis</u>.grey,6; yellowish, 1; 0,26.
  <u>optical character</u>.transparent,2; opalescent, 4; sebaceous, 1; 0. 26.
<u>Agar colony</u>.
  <u>form</u>. round, 26; filamentous, 1; 0, 6.
  <u>elevation</u>.flat,24; raised, 3; 0,6.
  <u>edge</u>.entire, 24; lobed, 3; 0, 6.
  <u>internal structure</u>. homogeneous, 2; granular, 24; grumose, 1; 0,6.
<u>Milk</u>.
  <u>coagulated</u>. +,2; 0, 31.
  <u>liquefaction</u>. 0,33.
  <u>reaction</u>.acid,7. alkaline,19; terminal alkalinity,7.

Summary of Cultural Characteristics.

It has been shown that of all the factors studied in this portion of the investigation, the proteid constituents, (and glycerine) are the only ones causing constant changes in growth forms- yet variations, due in part to the individual idiosyncrasies of the organisms, partly due to changes in the media too subtle for detection, do occur.

Yet one must rely upon certain cultural characteristics for separation of bacteria from each other.

The great value of cultural characteristics is to give a general idea of,-1- the characters of the colonies of particular species of bacteria, and -2- to furnish descriptive pictures of the various organisms upon the cultural media in common use.

While the cultural characteristics are similar, and even identical in certain groups of bacteria, this very point is valuable: one becomes familiar with the features common to the groups, and one can recognize members of one group in the presence of representatives of other groups.

This method is of fundamental importance in isolating

previously described organisms from a mixture of bacteria.

In general, one should study the cultural features upon various media, and indicate their limits of variation. Descriptions of such organisms, together with the morphological and biochemical characters described in the next part furnish a series of observations which will definitely define (classify) a given organism.

Work of this sort is valuable in proportion to the attention which is paid to the various details described in the preceeding chapters.

Careful attention to these points, particularly to the variations which may occur due to the differences in media, and variations in interpreting the various cultural phenomena would diminish greatly the number of 'species' incompletely described in bacteriological manuals.

Part. IV.

Morphology.

The bacteria are among the simplest of living things, and their morphology is correspondingly rudimentary.

Leaving out of consideration the higher bacteria, the bacterial cells are minute spheres, rods or spirals of protoplasm, enclosed in a more or less sharply defined limiting membrane, which may be demonstrated in many instances by placing the bacteria in hypertonic solutions i.e. solutions of various chemical substances which have relatively high osmotic pressures compared with the bacterial cells.

The membrane referred to is semi-permeable, allowing the passage of water in or out, but practically preventing the diffusion of the dissolved substances to any considerable extent.

If one immerses a bacterial cell in such a solution, water will be withdrawn from the cell-contents, and the latter will become shrunken, leaving the cell wall as a sharply defined, delicate membrane which retains its form in spite of the contraction of the cell protoplasm away from it.

The ease with which osmosis takes place varies greatly in [illegible]

the conclusion that the character of the limiting membrane varies; further evidence of this variation is shown by the avidity with which certain bacteria take up stains, and the difficulty with which other forms are decolorized when once the stain has penetrated. This may be due, in certain groups to definite chemical substances, as a sort of wax in the tubercle bacillus.

Many bacteria are quite resistant to heat, and others remain attached to one another after division, showing again differences in the character of the membrane surrounding them.

Morphology is the basis of any logical classification of living things, and even in the bacteria one finds certain characters which are of sufficient importance and constancy to furnish a means of primary division into families and subfamilies.

The geometrical arrangement of the Coccaceae, the flagellation of the Bacillaceae, the flagellation and flexuosity of the Spirillaceae are examples of such morphological characteristics.

In addition to the formation of spores, their position and relation to the form of the rod, and their method of germination are of great assistance in [illegible]

illaceae, e.g. the Subtilis group.

Spores have not been demonstrated, except in rare instances, in the Coccaceae and Spirillaceae.

Another characteristic- the method of reproduction in the Bacteriaceae- has received a new impetus through the work of Hill upon the 'hanging block' (Boston Board of Health Report, 1902). and also seems to be of prime importance in certain groups, particularly the Bacillus diphtheriae group, where many of the members 'snap' instead of 'slipping', the common method in bacillus coli, and many other common bacteria.

It will be necessary to discuss the morphological characteristics of the bacteria in detail.

Flagellated (motile) forms occur in each of the three great families of the bacteria, and this characteristic will be discussed first.

Many bacteriologists will not agree that flagellation is of sufficient importance to be used for the division of bacteria into primary groups, although Migula and [illegible] recognize this characteristic is of prime importance in classification.

The trouble seems to be that the results obtained are

not constant. The writer has devised a procedure whereby one may stain flagella with a high percentage of efficiency, and finds that with the same flagellated species studied, the results are very constant, and in every way suited for bacterial diagnosis and classification.

The method (Kendall, Jour. App. Microscopy, Vol. [illegible]) consists, not in a new stain, but in the preliminary treatment of the bacteria before staining.

Hitherto the great trouble has been that excessive handling has removed many of the flagella, and the results have been inconstant. By means of the method described below, students have succeeded in demonstrating satisfactorily and with comparative ease, the flagella upon many species of bacteria.

The method consists in inoculating into a tube of sterile water an amount of organisms from a fresh agar slant enough to produce a faint, but distinct turbidity in the upper third of the column of water.

The organisms are removed preferably with a loop in such a way that the wire is laid down, not dragged, upon the slanted surface, then introduced into the tube of water, and enough of the organisms allowed to float off to cause the desired turbidity.

One must not stir either the culture or the tube in which the organisms are finally placed because the flagella are very delicate, and any violent motion will remove them.

If one follows these directions carefully, the bacteria will be transferred from one tube to the other with a minimal amount of handling.

The tube of water containing the organisms is now incubated at the <u>optimum</u> temperature of the species from one to several hours, depending upon the rapidity of growth of the organisms.

One hour usually suffices for <u>Bacillus coli.</u>

Anaerobes may be grown anaerobically; care should be taken to boil and cool rapidly the water before using for such cultures to remove all dissolved oxygen.

During the incubation period there is a certain amount of reproduction, and non-flagellated forms sink to the bottom of the tube, because their organs of locomotion are gone; as a rule, only motile (flagellated forms) remain in suspension.

The water, free from albuminous material, washes, so to speak, the bodies of the bacteria, leaving them free from foreign matter, and in good condition to stain.

When one has obtained the organisms in this form, they are ready to be transferred to the cover glass for staining.

Perfectly clean cover glasses must be used; one removes a large drop of suspension from the upper portion of the tube containing the bacteria and deposits it upon the centre of the glass by touching the loop gently, then removing it immediately. A drop will adhere to the slide, and if this quantity is not enough, the process may be repeated, being careful as before not to disturb the bacteria by mixing with the loop.

If one follows these directions carefully, and has an emulsion of bacteria transferred to the slide with a minimal amount of handling, the bacteria retain their flagella, and are in the best possible condition for staining.

The film may be dried in the air, at 37° or over the water bath, depending upon the nature of the organism, and then fixed by passing once through the flame.

The preparation is now ready for staining, and any of the flagella stains may be used. The writer has found Pitfield's stain gives the most satisfactory results.

It must be remembered that this procedure is only t

with the preparation of the bacteria for staining, and that no method can perfect the staining technique of the individual. Faulty technique, not errors in the method are the ultimate source of error; this method can only make the conditions favorable. The final result depends upon the skill of the operator.

This method has yielded excellent results in the class room, and it is hoped that it will simplify the study of the flagellation of bacteria, particularly because this characteristic is of great importance in bacterial research.

Reproduction of Bacteria.

At the present time there are four methods for studying the reproduction of the bacteria;

1- Portions of fresh agar cultures are removed and stained. By studying the arrangement of the organisms with particular reference to their planes of division, one may get some idea of the mechanics of reproduction. This method is not at all satisfactory, and is rarely used.

2- Bacteria are studied in hanging drop preparations. With non-motile organisms it is possible to learn much about their method of growth, but of course the idea is practically worthless for motile forms.

3- Dr. [illegible] has used an agar hanging drop. This method is very good where observations are not to be extended over many days, and is interesting because it was the idea upon which Hill based his 'hanging block', the fourth method. The hanging block consists of a small square of agar cut from a sterile agar plate, upon the surface of which is spread a thin emulsion of the kind of bacteria to be studied. A cover glass is placed upon the side containing the bacteria, which imprisons the organisms between the upper surface of the agar and the cover glass. Organisms are practically free to grow in a horizontal plane, but are inhibited in the vertical plane. The block is suspended in a warm stage, and the edges of the cover glass sealed to the stage to prevent evaporation. One may make observations with the oil immersion lens, and extend such observations over two or three days if necessary. If one have a mechanical stage, one may get the rectangular coordinates of several organisms, and turn from one to another and return to any given bacterium at will. Continuous observations have shed much light upon the mechanics of colony formation, and the method furnishes bacteriologists with an extremely simple means of studying

this important problem.

Method of studying reproduction of bacteria.

A minute portion of culture from a fresh agar slant is emulsified with broth in such proportions that a field as observed with the oil immersion objective will contain only six or seven organisms.

An oese of this emulsion is spread upon the surface of a block of agar of suitable size, and a clean cover glass placed upon the emulsion. The agar will adhere to the coverglass, and if one wishes to prevent lateral movement, one may seal the agar more firmly to the cover glass by the use of a heated needle. The agar is melted, and when it solidifies, it clings closely to the coverglass. As a rule, only two edges need be melted in this manner. The block is suspended in the warm stage, and the immersion objective focussed upon the organisms.

The anaerobic bacteria may be grown quite as readily as aerobes by the use of inert gases in the stage.

One ought to illuminate the bacteria in such a manner that one may use the camera lucida; the mere observation of the reproduction is not enough for accurate bacterial work. The camera allows one to draw the organisms from the

beginning of the experiment until the field is so overgrown that further observation is impossible.

The changes in size are very interesting, and bring out some points which hitherto have not been emphasized by bacteriologists.

The first change consists in the gradual enlargement of the cell about to divide. Not only does the long diameter become greater, as previous investigators have found, but the short diameter also elongates. The organism is sometimes almost twice as long, and twice as thick as the cell in its initial condition.

Next a short, slight indentation occurs at the point of future plane of division, and this gradually becomes extended as a dark line which eventually cuts the cell into two equal halves.

The resulting daughter cells during the first two or three generations are usually larger than the original, parent cell before it began to enlarge; soon, however, there is a gradual diminution in size, until finally the organisms become of approximately the same size as the original cell. In certain cases where preliminary cultivation has not been made, the first cell was very small indeed:

it took longer for such cells to attain their usual size before division, and the reproduction was less rapid than in bacteria which were in active vegetative reproduction at the beginning of the observation.

It is impossible to witness this phenomenon without comparing it with the observations made by various observations concerning the variability in size of bacteria: Fischer (loc. cit) mentions giant and dwarf cells occurring in the same cultures, and others have noted differences in size of bacteria in the same culture at different times.

It is of importance to note that if one looks at a preparation of bacteria under the high powers, it is not the variation in size, but the mean dimensions which attract one's attention in other words, one does not note the giants and dwarfs, but carries away a mean mental picture of the culture.

It is only on careful scrutiny that one will see the comparatively great variations in size.

In all probability, the explanation of the variations in size of bacteria noted by previous observers are in large part due to the comparative freshness of their cultures when such observations were made.

These facts completely overthrow the old idea that each organism had a certain fixed dimension, which was incapable of any considerable change.

It is almost certain that the explanation of the increase of size is due to removal of the waste products, which is brought about by transferring to fresh media. In fact one may almost find a direct relation between the relative increase of size (up to a certain limit) and the relative absence of effete products. One may bring about the same change by diluting the medium with sterile water in the case of fluid media. Under these conditions, the organisms begin to grow with renewed luxuriance, (showing that bacteria do not as a rule use up the food supply) and there is coincidently an increase in size of the bacteria.

The meaning of these observations is very evident; by frequent transfers upon fresh media, the old organisms are replaced by their descendants which are in the very best vegetative condition: they are larger than the original organisms and grow more rapidly. Their average size may be almost twice as great.

Fischer (loc. cit, p. 45) gives some very important data upon this subject from a slightly different point of view; a culture of the cholera spirillum contained (estimated) 22,850,000,000 living organisms. After a lapse of 4 hours, 4800 [illegible] 000 had died; after 40 hours, only 10 [illegible] were living.

We have then as a result of increasing waste products in the same culture, a rapid diminution in numbers, accompanied by a diminution in size of the organisms.

It is not remarkable, then, that bacteria kept upon agar slants for a month or more, when inoculated directly upon the usual laboratory media, do not give all their characteristic reactions. This explains fairly well the lack of constancy which is a feature of bacterial descriptions.

The constancy of results obtained by a few investigators is in all probability due to a careful attention to this same factor-- careful preliminary cultivation.

The flagellation of the organisms studied in this investigation showed the constancy of this characteristic for purposes of classification; cultures of Bacillus typhosus, coli and prodigiosus were invariably peritrichic. The number of flagella varied, but they were always placed around the body of the bacilli.

The flagella upon Bacillus [illegible] were invariably mono-trichic; in one of the 17 cultures two polar flagella were found, but this agrees perfectly with the classical description. As a whole, the study of the flagellation was very satisfactory; no particular difficulties were met with in demonstrating this characteristic.

Spore Formation and Germination.

None of the organisms studied in this investigation produced spores- but the 'hanging block' method is particularly valuable for the study of their formation and germination.

One makes a thin emulsion of bacteria containing spores and prepares a hanging block in the usual manner. After a longer or shorter time the spore membrane becomes less refractile, and soon the germinating rod pushes through, either at the pole or equator of the rod.

The position of the spore, the swelling of the rod at sporulation, and the method of germination. (equatorial or polar) are characteristics of importance in the classification of certain groups of bacteria, particularly the Subtilis group.

<u>Classification of Bacteria.</u>

In the preceeding portions of this investigation,

the cultural, morphological and biochemical characteristics of certain bacteria have been studied, their relative constancy, and applicability for bacterial classification discussed.

To summarize briefly what has been shown, we have seen that the form, flagellation and geometrical arrangement of bacteria are the important morphological characteristics; liquefaction of gelatin and fermentation of carbohydrate the biochemical characteristics which one may rely upon for classification.

Not only must one have constants for use in the division of bacteria into final groups, but one must distinguish bacteria from one another when they exist side by side in mixed cultures.

This leads to two great classes of bacterial characteristics;

1- those upon which one must rely for the separation of bacteria from one another-cultural appearances (reactions). These reactions are valuable for obtaining pure cultures but are subject to variations which have been discussed in Part III. The importance of <u>average</u> or <u>mean</u> descriptions of cultural characteristics in our systematic bacteriology becomes apparent in this connection, because it is

[illegible] [illegible] upon which one [illegible] [illegible] [illegible]
[illegible].

It is these [illegible] reactions which are relatively constant - characteristics which one must rely upon for the division of bacteria into groups.

The logical method of procedure in isolating an organism, then, is first to know its habitat, and seek it there. second- to isolate a series of colonies from plates: colonies which resemble the colony described as typical for that 'species'.. third- to be certain of the purity of the organisms, and give them the usual preliminary cultivation to insure an active vegetative condition. fourth- to place such organisms in their proper groups by determining the form, flagellation, liquefaction of gelatin and fermentation of dextrose. fifth- to apply such confirmatory tests as shall finish the list of reactions considered necessary for a bacterial description, and then establish the identity of the organism.

In part I will be found a short description of the method used for recording the descriptions of bacteria.

The morphological classification of bacteria, herewith presented, is somewhat unique; the flagellation is made a definite criterion, next in importance to the form of

the bacterial cell.

Bacteriaceae. *

10. Coccaceae. cells spherical in the free state, not
elongated in any direction until division.

Atricha, without flagella.

11. Streptococcus, Billroth. planes of fission
parallel, giving rise to chains.
12. Micrococcus, Cohn. planes of fission without
definite sequence.
13. Tetracoccus, Welch. two planes of fission alternate and at right angles,giving rise to tetrads.
14. Sarcina; Goodsir. three planes of fission at
right angles to each other.

Tricha. Kendall. flagellated forms.

15. Planococcus, Migula. motile,micrococcus.
16. Planosarcina, Migula. motile sarcinae.

20. Bacillaceae. cells elongated, cylindrical; longer
diameter increased before division.

Atricha, Kendall. non-flagellated.

* 21, Bacterium, Ehrenberg. non-motile.

Tricha, flagellated forms.

22. Bacillus, Cohn. flagellation peritrichous.
23. Monotrichus, Kendall. polar flagellum,(rarely
two polar flagella.
24. Lophitrichus, Kendall. tuft of polar flagella.

30. Spirillaceae. cells cylindrical,spirally twisted.

Atricha, Kendall. non-flagellated forms.

31. Spirosoma, Migula. cells rigid,without flagella.
32. Spirochaetae, Ehrenberg.cells without flagella,
motility by flexuous membrane.

Tricha.

33. Microspira, Schroeter. one,rarely two polar
flagella.
34. Spirillum. Ehrenberg. tuft of polar flagella.

* This grouping does not include the higher bacteria.

It will be noticed that the term Tricha and Atricha has been introduced; this adds homogeneity to the system, making flagellation a definite basis of classification.

Attention is also called to the introduction of the terms mono- and lophotrichous in place of the term Pseudomonas of Migula. Migula believes the bacilli with polar flagella are not as important individually as the peritrichous bacilli, and includes both genera in one group. Both the term Pseudomonas and the conception of the genus are not correct. This becomes all the more interesting when one notes that the spiral bacteria are divided into two groups, having one and tuft of polar flagella respectively.

The further division of bacteria cannot be made upon a morphological basis; one must rely upon physiological characteristics.

Of these the liquefaction of gelatin and the fermentation of dextrose are the most important and most constant.

By the use of morphology, and the biochemical characters mentioned, one may divide bacteria into types, but even these types are not of sufficient specificity to serve the purposes of a bacterial classification.

One must proceed further; at the same time one has practically exhausted the characteristics which are constant, and common to the majority of bacteria.

Further subdivision depends upon characteristics which

are rather varied; characteristics important in one group will be inconstant and without importance in other groups. The fermentation of carbohydrates is of great importance in the Colon and Paracolon groups, and without significance in the Subtilis group, and spore formation, highly important in the latter group, is of no value in the former group.

Before outlining the general method of the ultimate classification of bacteria into well defined small groups, it will be well to explain briefly the method of using the morphological and biochemical classification for division into types.

The characteristics employed are, form, flagellation, liquefaction of gelatin and fermentation of carbohydrate. In addition, the prevailing color of the growth upon the agar slant has been made provisionally.

It will be remembered that those bacteria, for example, whose prevailing color was white, were designated by numbers running from 10 to 18 inclusive. red forms ran from 30 to 38, and so on. In each instance, the predominating color came first, and the various tints were designated by the various second numbers. gray being 11, red-brown 35.

Likewise, two digits were appended to each of the morphological divisions; so that the Coccaceae were represented by digits running from 11 to 15, the Bacteriaceae from 21 to 24, and the Spirillaceae from 31 to 34.

In this classification, the initial digit represents the <u>form</u>, the second digit the flagellation or geometrical arrangement of the cells, so that 11 indicates a Streptococcus, 22 a peritrichicly flagellated bacillus, etc.

If one can in like manner designate the various reactions of bacteria upon gelatin and dextrose, one may graphically (numerically) designate any type of bacterium. Such a representation will greatly facilitate the classification of bacteria, and render their location in literature (serial tables) a matter of great ease.

The possible combinations of liquefaction of gelatin and fermentation of destrose are indicated below.

	Liquefaction of gelatin.	Fermentation of carbohydrate.	
		acid.	gas.
1.	-	-	-
2.	-	+	-
3.	-	+	+
4.	+	-	-
5.	+	+	-
6.	+	+	+
7. *	?	-	-
8. *	?	+	-
9. *	?	+	+

The complete TYPE NUMBER consists of four digits; the first two refer to the morphology (form, flagellation,) the third to the liquefaction of gelatin and fermentation of dextrose, the fourth to the predominating color upon the agar slant.

Acting upon the suggestion of Mr. Whipple of the Mt. Prospect Laboratory, the decimal point is inserted between the first two (morphological) and the last two (physiological) digits.

Two examples will make the use of the TYPE NUMBERS plain;

22.53. refers to a bacillus, (see morphological table,) having monotrichic flagellation; liquefies gelatin and produces acid in dextrose (see table upon preceeding page) and whose predominating color upon the agar slant is red. (see table of color scheme.)

11.31. a coccus, growing in chains (hence streptococcus) with no flagella, does not liquefy gelatin, growth white upon agar.

One can arrange the descriptions of bacteria upon tables, placeing appropriate group numbers in order, and look up any organism belonging to that group in a moment.

The further use of the numerical scheme for recording the cultural characteristics, used throughout this investigation in the tables, will make the complete identification of a given species with another, written, description, the work of a moment. All one has to do is to write the characteristics in terms of their numerical equivalents in the proper order, and turn to the proper type number. Identity of symbols will at once mean identity of descriptions.

The final division of bacteria into groups. No general statements can be made, but a few groups are appended that the method may be clear. The type number will be placed first, together with the name of the group, and additional characteristics necessary for placing the organisms in their proper groups will be added.

Colon group. type number [illegible].

Lactose fermented with the production of gas. Milk coagulated.

Paracolon group. type number, [illegible].31.

Gas in hexoses, but not in biozes. Milk; acid, then alkaline, terminal alkalinity.

Typhoid group. type number, [illegible].21.

No gas in carbohydrates. Acid produced.

Milk, three types; acid, alkaline, and terminal alkalinity.

Bacillus subtilis group. type number, 22.31.

Ferments no carbohydrate.

Milk coagulated, liquefied, alkaline.

Spore- central in rod, rod not swollen at sporulation.

Germination equatorial.

Bacillus cloacae group. type number, 2[illegible]. 61.

Ferments lactose with gas production.

Milk coagulated, liquefied, acid.

Micrococcus albus group. type number, 1[illegible].51.

Acid in dextrose.

Milk coagulated, acid.

Microspira comma group. type number, 3[illegible].51.

Acid in dextrose.

Milk coagulated, acid.

Nitroso-indol produced.

Part V. General Summary.

Use of the 'Numerical or Decimal' System of recording the characteristics of bacteria.

The [illegible] symbol is a convenient method for classifying bacteria into 'types': the four digits comprising this symbol are divided into two groups of two digits each. The first digit indicates the form of the organism, (coccus, bacillus, spirillum): the second the flagellation or geometrical sequence of division, and the resulting geometrical configuration of the daughter cells in space, (streptococcus, tetracoccus, sarcina: mono, peri or lophotrichic flagellation). These two numbers define the morphological characteristics of the organisms.

The third digit indicates the liquefaction or non-liquefaction of gelatin, and the fermentation of dextrose, (gas or acid production).

The fourth digit describes the prevailing color upon the agar slant.

The above characteristics are common to all bacteria, are very constant in their occurrence, and divide bacteria into groups of approximately equal size and value.

Further division into groups depends upon character-

[illegible] not common to all bacteria, but characteristic for each group. Such divisions are indicated in the last portion of Part IV.

The cultural characteristics of bacteria are defined; 1- in definite, standard terms: terms chosen with reference to two important factors- specific enough to define sharply and definitely any reaction of any bacterium upon any of the ordinary media, yet general enough to allow for the slight variations characteristic of living organisms.

To each of these standard, definite terms, numerals are assigned- in general the simplest characteristic is represented by the lowest numeral, and increase in complexity of characteristic (also in infrequency of occurrence) is coincident with increased denomination of the numeral.

A standard table or chart (as charts used in this investigation) is provided, with the observations usually made upon each kind of medium: agar slant, gelatin stab, plates, etc.

One makes the proper cultural observations of the organism under consideration and writes the empirical expression for the characteristic in the appropriate column.

biochemical reactions are indicated (+) when a characteristic is positive, (-) when it is negative, or [illegible] [illegible] [illegible].

The signs $\pm$ or $\mp$ are used; the first designates a positive reaction followed by a disappearance or negativity of the characteristic: the second a negative or absent reaction followed by a positive reaction.

These signs are particularly useful in tabulating the reactions of various media during, and after bacterial growth. The first sign, ($\pm$) indicates an acidity followed by neutrality or alkalinity: the second, ($\mp$) an alkalinity followed by acidity.

This system of tabulation, while apparently complicated, has been used in a class of beginners in bacteriology, with satisfactory results. There is an immense saving of time in writing the results, and the terms and their symbols are memorized rapidly.

Another form is appended, using the standard terms instead of their symbols (numerical symbols). While it is less concise than the numerical table, yet one may use it with a minimal expenditure of time. All the ordinary characteristics of bacteria are provided for, and [illegible]

arrangement allows one to economize space.

The advantage of using standard terms is admirably illustrated by Professor Chester in his Manual of Determinative Bacteriology (p;17); the terms used in this work are those first proposed by him, with a few additions and changes for increased clearness and comprehensiveness.

Classification of Bacteria.

The classification presented here is in many respects a departure from older systems; it recognizes several factors which do not appear in other systems, and is made as far as possible <u>homogeneous</u>. Any artificial classification must be empirical to a certain extent, and it is well to choose ones criteria with the view of homogeneity if possible.

The classification will be outlined again, briefly; calling attention to the salient points.

It is divided into two parts, a Morphological and a Physiological portion.

Morphology;

1- Form of the organism.

1- Flagellation; bacteria are divided into the <u>Trichia</u>, or flagellated forms, and the <u>Atrichia</u> or non-flag-

[illegible] forms.

Further morphological subdivisions depend upon the geometrical arrangement of the daughter cells, and in the spore forming groups upon the form of the spore and rod, and the method of germination of the spore.

Physiological.

1- Liquefaction or non-liquefaction of gelatin.

2- Fermentation of dextrose with the production of gas or acid.

The above criteria are common and constant for all bacteria.

Further subdivision depends upon characteristics not common to all bacteria, but constant for certain groups.

It will be impossible to summarize satisfactorily the methods and precautions described in Part I- mention should be made of the importance of preliminary cultivation in accurate descriptive work upon bacteria.

Part II; the results of the investigation upon the investigation of different strains of the same organism upon the same media show that aside from the individual peculiarities of cultures of the same species, the bacteriologists agree very well upon the characteristics which are made use in identifying a given species.

of all the cultures submitted, all except one proved to be the species corresponding with the label.

Part III; the concentration of the media affected, in general, the <u>extent</u> to which the bacterial growth spread.

The reaction of the medium, within the limits used in bacterial research, was without effect upon the cultural or biochemical characteristics of common, vigorously growing bacteria. In certain forms, the reaction would play an important part, but in general such forms are not likely to be met with in ordinary work.

Moisture is necessary for the longevity of bacterial growth; this must be in the general environment as well as in the media upon which organisms are grown.

The character of the albuminous substances, as well as glycerine affects the chromogenic properties, and not the cultural and morphological characteristics of bacteria. This is particularly marked in the chromogenic bacteria.

There is a 'personal factor' in making media and in interpreting the cultural characters of bacteria.

Liquefaction of gelatin and the fermentation of dextrose are the most constant common physiological characters of bacteria, and the most suited for division of the bacteria into groups.

Other biochemical characters, constant and important in certain groups, are inconstant and unimportant in other groups, hence suited for the identification of the final subdivisions of bacteria, but ill suited for general classification.

Cultural reactions, like any growth forms, are subject to slight variations; variations due to subtle changes in the composition of the media, and upon individual idiosyncrasies of the organisms themselves.

One does, however, detect a <u>mean</u> or <u>average</u> appearance, which not specific as a rule for individual species, are of paramount importance in isolating certain groups of bacteria from other groups: for obtaining pure cultures.

Part IV; the bacteria have in addition to their form, and flagellation certain other morphological characters which, although little known at present, will be of immense importance in bacterial classification. The method of reproduction, and the method of colony formation are the most important of these.

Capsule formation and staining reactions - more chemical in nature, are not without value in bacterial diagnosis.

In conclusion, the writer wishes to thank the following gentlemen for their great kindness in facilitating [illegible]

furthering this investigation;

Dr. Welch for his supervision of the work, for laboratory privileges, and for his hearty cooperation without which this investigation would have been impossible.

Drs. [illegible] and Ford, of the University staff, for their many kindnesses in furnishing cultures, and material.

Drs. Hill, Theobald Smith, [illegible], Abbott, Wesbrook, Novy, Jordan, [illegible], Stokes, [illegible], Flexner, for furnishing cultures, and data.

To Professor Chester for valuable information and data concerning the use of standard terms, etc.

To the members of the class of 1906, of the Medical School for their cooperation in the work upon 'personal factor' of cultural interpretation, and also to the laboratory force of the Pathological Laboratory for their many courtesies.

Baltimore, 1904. Arthur Isaac Kendall.

Appendix.

Composition and reaction of media used in this investigation.

Medium.	% agar or gelatin.	% peptone.	reaction.	other ingredients.
Agar 1.	1.[illegible]	1	+0.1	meat extract, 2.5 grs. glycerine, 5 %.
Agar 2.	1.5	1	+0.1	meat extract, 2.5 grs.
Agar 3.	1.5	1	0.0	" " 2.5 "
Agar 4.	1.0	1	+ 1.[illegible]	meat juice.
Agar 5.	1.0	-	+ 0.1	ox serum, 10 %.
Agar 6.	1.5	1	+ 0.1	meat extract, 2.5 grs.
Agar 7.	1.2	1	+ 0.5	---
Agar 8.	1.2	1	+ 0.7	5 % glycerine.
Agar 9.	1.2	-	0.0	5 % starch.
Agar 10.	1.0	1	+ 0.[illegible]	10 % ox serum.
Agar 11.	1.5	1	+ 0.2	meat extract, 2.5 grs.
Agar 12.	1.5	1	+ 0.2	do.
Agar 13.	1.5	1	+ 0.1	do.
Agar 14.	1.[illegible]	1	0.0	do.
Agar 15.	1.[illegible]	1	+ 0.4.	

Gelatin No.	Gelatin.	Peptone.	Reaction.	[illegible]
Gelatin 1.	16	1	- 0.3	2.5 grs.
Gelatin 2.	16	1	- 0.1	[illegible].5 grs.
Gelatin 3.	8	1	+ 1.0	meat juice.
Gelatin 4.	16	1	+ 1.2	meat juice.
Gelatin 5.	15	1	0.1	extract, 2.[illegible] grs.
Gelatin 6.	15	1	+ 0.5	do.
Gelatin 7.	15	1	0.4	do.
Gelatin 8.	15	1	+ 0.2	do.
Gelatin 9.	15	1	+ 0.[illegible]	do.

I, Arthur Isaac Kendall, was born in Somerville, Massachusetts, May 7, 1877. I attended the regular public primary, grammar and High Schools in that city, and at the age of 18 was admitted to the freshman class of the Massachusetts Institute of Technology in 1895. Owing to a protracted illness, I was not able to complete the first year at that institution, and was compelled to take an extra year. At the beginning of my sophomore year I decided to take the Biological Course in preference to any other, and worked in this department under the supervision of Professor Sedgwick, until I was graduated, in 1900. I cannot do better than to take this opportunity to express my heart-felt gratitude to Professor Sedgwick for his many kindnesses to me while I was a student in his department. His goodness was not confined to my undergraduate career, but was again manifested in [illegible], when he assisted me to obtain the Swett Fellowship from that institution, so that I could continue my studies at Johns Hopkins, two years later.

After graduating from the Institute of Technology, I was employed first by Mr. Simeon C. Keith of the firm of Hood and Sons, milk contractors; my duties consisted in getting up pure cult-

ures of bacteria for ripening cream and cheese, and also to inspect bacteriologically the various dairies from which they were supplied with milk.

In September of that year, 1900, I was appointed Chief Chemist and Biologist for the Cinclare Sugar Plantation in Louisiana, and I entered upon my new duties in October of that year. I remained there during the grinding season, and left in January to become assistant to Mr. R. S, Weston, Resident Expert for the New Orleans Sewerage and Water Board. While there I controlled under Mr. Weston's direction, the bacteriological work of the station, both routine and experimental.

In May, 1901, I came to Lawrence, Mass, as assistant Bacteriologist for the Lawrence Experiment Station, where I spent a year in studying the purification of Sewage, and Sewage disposal.

In June, 1902, I was appointed Swett Fellow of the Institute of Technology, and spent the time between that period and September, the time I entered Johns Hopkins University, working with Dr. H.W.Hill, Director of the Bacteriological Laboratory of the Boston Board of Health.

In September, 1902, I began work at Johns Hopkins, and [illegible] bacteriology and physiological chem-

try as well as working upon my dissertation. In addition to the work mentioned above, I am engaged in preparing material for Drs. [illegible] and Jones for an investigation upon certain nucleo-proteins of the pancreas and thymus. In June, 19[illegible], I was appointed a fellow of the Rockefeller Institute, and worked upon the organisms of summer dysentery from June until the latter part of September.

Through the kindness of Dr. Welch, I was mentioned as a candidate for the Fellowship in Pathology and Bacteriology of the Johns Hopkins University, obtained the appointment, and have spent the present year in that capacity working upon my dissertation, and demonstrating in bacteriology.

In conclusion I cannot do otherwise than to thank Dr. Welch for his many kindnesses to me, both in obtaining for me the fellowship, and in directing my work since I have been enrolled at this university.

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AGAR COLONY	MILK.	FERMENTATION	NITRATE.			Pathogenesis—Human.	REMARKS.
							Table 1.
							Nitro Indol formed

FOLD OUT

FOLD OUT

Table 2

Phenol.	Pathogenesis—Human.	REMARKS.
		Widal
	+	1/50 +
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+
	+	+

* distinct trace of indol in all cultures

NAME.	INVESTI[illegible]	AGAR [illegible]OLONY.			MILK.				FERMENTATION					NITRATE.							REMARKS.
		[illegible]ny.		[illegible]e.							[illegible]se.	[illegible]d Arm.	[illegible]xtrose.					[illegible]hide.		[illegible]Human.	

Table 3

a[illegible]

FOLD OUT

FOLD OUT

Phenol.	Pathogenesis—Human.	REMARKS.
		-Table 4.

+ litmus decolorized

Table 5

NAME.	INVESTIGATOR. Day of observation	Agar No.	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores.	Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.
…acillus coli 1	10	1	1											1	/	/	11	/	22		—	2	/	—	22	4
		2	2											1	/	/	11	/	22	—	—	2	/			
		3	3											1	/	/	11	/	22		—	2	/			
		4	4											5	/	/	10	/	22	—	—	2	/			
		5	5											1	/	/	11	/	22	—	—	2	/			
		6												1	/	/	11	/	22	—						
2	10	1	1											1	/	/	11	/	22		—	2	/	2	22	4
		2	2											1	/	/	11	/	22	—	—	3	/			
		3	3											1	/	/	11	/	22	—	—	2	/			
		4	4											9	/	/	10	/	22	—	—	4	/			
		5	5											1	/	/	11	/	22	—	—	3	/			
		6												1	/	/	11	/	22	—						
3	10	1	1											1	/	/	11	/	22	—	—	2	/	2	22	4
		2	2											1	/	/	11	/	22	—	—	2	/			
		3	3											1	/	/	11	/	22	—	—	2	/			
		4	4											9	/	/	10	/	22	—	—	2	/			
		5	5											1	/	/	11	/	22		—	2	/			
		6												1	/	/	11	/	22	—						
4	10	1	1											1	/	/	11	/	22	—	—	2	/	2	22	4
		2	2											1	/	/	11	/	22	—	—	4	/			
		3	3											4	/	/	11	/	22	—	—	2	/			
		4	4											5	/	/	10	/	22	—	—	2	/			
		5	5											1	/	/	11	/	22	—	—	2	/			
		6												1	/	/	11	/	22	—						
5	10	1	1											1	/	/	11	/	22	—	—	4	/	—	22	4
		2	2											1	/	/	11	/	22	—	—	4	/			
		3	3											4	/	/	11	/	22	—	—	4	/			
		4	4											5	/	/	10	/	22	—	—	5	/			
		5	5											1	/	/	11	/	22	—		4	/			
		6												1	/	/	11	/	22	—						
6	10	1	1											1	/	/	11	/	22	—	—	4	/	—	22	4
		2	2											4	/	/	11	/	22	—	—	2	/			
		3	3											1	/	/	11	/	22	—	—	4	/			
		4	4											9	/	/	10	/	22	—	—	5	/			
		5	5											2		/	11	/	22	—	—	4	/			
		6												1	/	/	11	/	22	—						

Table 6

T

NAME.		INVESTIGATOR. Day of observation	Agar No	Gelatin No	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores.	Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.
cillus coli	7	10	1	1											1	1	1	11	1	22	–	–	2	1	–	22	4
			2	2											9	1	1	11	1	22	–	–	2	1			
			3	3											4	1	1	11	1	22	–	–	2	1			
			4	4											1	1	1	10	1	22	–	–	4	1			
			5	5											2	1	1	11	1	22	–	–	2	1			
			6												4	1	1	11	1	22	–						
	8	10	1	1											1	1	1	11	1	22	–	–	4	1	–	22	4
			2	2											9	1	1	11	1	22	–	–	5	1			
			3	3											4	1	1	11	1	22	–	–	4	1			
			4	4											9	1	1	10	1	22	–	·	3	1			
			5	5											2	1	1	11	1	22	–	–	5	1			
			6												4	1	1	11	1	22	–						
	9	10	1	1											1	1	1	11	1	22	–	5	–	–	/	22	4
			2	2											1	1	1	11	1	22	–	5	–	–			
			3	3											1	1	1	11	1	22	–	5	–	–			
			4	4											9	1	1	10	1	22	–	5	1	T			
			5	5											1	1	1	11	1	22	–	5	1	T			
			6												1	1	1	11	1	22	–						
	10	10	1	1											1	1	1	11	1	22		–	2	1	–	22	4
			2	2											1	1	1	11	1	22	–	–	2	1			
			3	3											4	1	1	11	1	22		–	2	1			
			4	4											1	1	1	10	1	22	–	–	4	1			
			5	5											4	1	1	11	1	22	–	–	2	1			
			6												1	1	1	11	1	22	–						
	11	10	1	1											1	1	1	11	1	22	–						
			2	2											1	1	1	11	1	22	–						
			3	3											1	1	1	11	1	22	–						
			4	4											5	1	1	10	1	22	–						
			5	5											1	1	1	11	1	22	–						
			6												1	1	1	11	1	22	–						
	12	10	1	1											1	1	1	11	1	22	–						
			2	2											1	1	1	11	1	22	–						
			3	3											1	1	1	11	1	22	–						
			4	4											1	1	1	10	1	22	–						
			5	5											1	1	1	11	1	22	–						
			6												1	1	1	11	1	22							

Table 6

T

NAME.		INVESTIGATOR.			TYPE NUMBER.	MORPHOLOGY.								AGAR.							GEL.			BROTH.			
		Day of observation	Agar No	Gelatin No		Diameter + 1 micron.	Form in Rod. Spores.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	Liquefaction—Form.	Line Growth.	Surface Growth.	Pellicle.	Turbidity.	Sediment.	Liquefaction
illus coli	7	10	1	1										1	1	1	11	1	22	–	–	2	1	–	22	4	
			2	2										9	1	1	11	1	22	–	–	2	1				
			3	3										4	1	1	11	1	22	–	–	2	1				
			4	4										1	1	1	10	1	22	–	–	4	1				
			5	5										2	1	1	11	1	22	–	–	2	1				
			6											4	1	1	11	1	22	–							
	8	10	1	1										1	1	1	11	1	22	–	–	4	1	–	22	4	
			2	2										9	1	1	11	1	22	–	–	5	1				
			3	3										4	1	1	11	1	22	–	–	4	1				
			4	4										9	1	1	10	1	22	–	–	3	1				
			5	5										2	1	1	11	1	22	–	–	5	1				
			6											4	1	1	11	1	22	–							
	9	10	1	1										1	1	1	11	1	22	–	5	–	–	/	22	4	
			2	2										9	1	1	11	1	22	–	5	–	–				
			3	3										1	1	1	11	1	22	–	5	–	–				
			4	4										9	1	1	10	1	22	–	5	1	T				
			5	5										1	1	1	11	1	22	–	5	1	T				
			6											1	1	1	11	1	22	–							
	10	10	1	1										1	1	1	11	1	22		–	2	1	–	22	4	
			2	2										1	1	1	11	1	22	–	–	2	1				
			3	3										4	1	1	11	1	22	–	–	2	1				
			4	4										1	1	1	10	1	22	–	–	4	1				
			5	5										4	1	1	11	1	22	–	–	2	1				
			6											1	1	1	11	1	22	–							
	11	10	1	1										1	1	1	11	1	22	–							
			2	2										1	1	1	11	1	22	–							
			3	3										1	1	1	11	1	22								
			4	4										5	1	1	10	1	22	–							
			5	5										1	1	1	11	1	22								
			6											1	1	1	11	1	22	–							
	12	10	1	1										1	1	1	11	1	22	–							
			2	2										1	1	1	11	1	22	–							
			3	3										1	1	1	11	1	22	–							
			4	4										1	1	1	10	1	22	–							
			5	5										1	1	1	11	1	22	–							
			6											1	1	1	11	1	22	–							

Table 7

NAME.	INVESTIGATOR.			TYPE NUMBER.	Diameter + 1 micron.	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	AGAR. Elevation of Growth.	AGAR. Lustre.	AGAR. Chromogenesis.	AGAR. Topography of Growth.	AGAR. Optical Characters.	AGAR. Viscosity.	GEL. Liquefaction—Form.	GEL. Line Growth.	GEL. Surface Growth.	BROTH. Pellicle.	BROTH. Turbidity.	BROTH. Sediment.
cillus Coli 1	4	1	1										9	/	/	11	/	22	~	-	2	/			
		2	2										9	/	/	11	/	22	~	-	2	/			
		3	3										9	/	/	11	/	22	~	-	2	/			
		4	4										5	/	/	10	/	22	~	-	2	/			
		5	5										9	/	/	11	/	22	~		2	/			
		6											9	/	/	11	/	22	~						
	7	1	1										9	/	/	11	/	22	~	-	2	/			
		2	2										9	/	/	11	/	22	~	-	2	/			
		3	3										9	/	/	11	/	22	~	-	2	/			
		4	4										5	/	/	10	/	22	~	-	2	/			
		5	5										9	/	/	11	/	22	~	-	2	/			
		6											9	/	/	11	/	22	~						
	10	1	1										1	/	/	11	/	22	~	-	2	/			
		2	2										1	/	/	11	/	22	~	-	2	/			
		3	3										1	/	/	11	/	22	~	-	2	/			
		4	4										5	/	/	10	/	22	-	-	2	/			
		5	5										1	/	/	11	/	22	~	-	2	/			
		6											1	/	/	11	/	22	~						
2	4	1	1										1	/	/	11	/	22	~	-	2	/			
		2	2										1	/	/	11	/	22	~	-	2	/			
		3	3										4	/	/	11	/	22	~	-	2	/			
		4	4										9	/	/	10	/	22	~	-	2	/			
		5	5										1	/	/	11	/	22	~	-	2	/			
		6											1	/	/	11	/	22	~						
	7	1	1										1	/	/	11	/	22	~	-	2	/			
		2	2										1	/	/	11	/	22	~	-	4	/			
		3	3										4	/	/	11	/	22	~	-	2	/			
		4	4										9	/	/	10	/	22	~	-	2	/			
		5	5										1	/	/	11	/	22	~		4	/			
		6											1	/	/	11	/	22	~						
	10	1	1										1	/	/	11	/	22	~	-	2	/			
		2	2										1	/	/	11	/	22	~	-	3	/			
		3	3										1	/	/	11	/	22	~	-	2	/			
		4	4										9	/	/	10	/	22	~	-	4	/			
		5	5										1	/	/	11	/	22	~	-	2	/			
		6											1	/	/	11	/	22	~						

NAME.	INVESTIGATOR. Day of observation	Agar No.	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores.	Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.
cillus coli 3	4	1	1											9	1	1	11	1	22	–	–	3	1			
		2	2											4	1	1	11	1	22	–	–	3	1			
		3	3											4	1	1	11	1	22	–	–	3	1			
		4	4											9	1	1	10	1	22	–	–	3	1			
		5	5											2	1	1	11	1	22	–	–	3	1			
		6												4	1	1	11	1	22	–						
	7	1	1											9	1	1	11	1	22	–	–	3	1			
		2	2											4	1	1	11	1	22	–	–	3	1			
		3	3											4	1	1	11	1	24	–	–	3	1			
		4	4											9	1	1	10	1	22	–	–	2	1			
		5	5											2	1	1	11	1	22	–	–	2	1			
		6	6											4	1	1	11	1	22	–						
	10	1	1											9	1	1	11	1	22	–	–	2	1			
		2	2											9	1	1	11	1	22	–	–	2	1			
		3	3											4	1	1	11	1	22	–	–	2	1			
		4	4											9	1	1	10	1	22	–	–	2	1			
		5	5											2	1	1	11	1	22	–	–	2	1			
		6												4	1	1	11	1	22	–						
4	4	1	1											4	1	1	11	1	22	–	–	3	1			
		2	2											4	1	1	11	1	22	–	–	3	1			
		3	3											4	1	1	11	1	22	–	–	3	1			
		4	4											5	1	1	10	1	22	–	–	3	1			
		5	5											4	1	1	11	1	22	–	–	3	1			
		6												4	1	1	11	1	22	–						
	7	1	1											4	1	1	11	1	22	–	–	3	1			
		2	2											4	1	1	11	1	22	–	–	3	1			
		3	3											4	1	1	11	1	22		–	3	1			
		4	4											5	1	1	10	1	22	–	–	3	1			
		5	5											4	1	1	11	1	22	–	–	3	1			
		6												4	1	1	11	1	22	–						
	10	1	1											1	1	1	11	1	22	–	–	2	1			
		2	2											1	1	1	11	1	22	–	–	4	1			
		3	3											4	1	1	11	1	22	–	–	2	1			
		4	4											5	1	1	10	[illegible]	22	–	–	2	1			
		5	5											1	1	1	11	1	22	–	–	2	1			
		6												1	1	1	11	1	22	–						

Table 9

NAME.	INVESTIGATOR. Day of observation	Agar No.	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	AGAR. Elevation of Growth.	AGAR. Lustre.	AGAR. Chromogenesis.	AGAR. Topography of Growth.	AGAR. Optical Characters.	AGAR. Viscosity.	GEL. Liquefaction—Form.	GEL. Line Growth.	GEL. Surface Growth.	BROTH. Pellicle.	BROTH. Turbidity.	BROTH. Sediment.	Liquefaction
acillus coli 5	4	1	1										1	1	1	11	1	22	—	—	3	1				
		2	2										4	1	1	11	1	22	—	—	3	1				
		3	3										4	1	1	11	1	22	—	—	3	1				
		4	4										5	1	1	10	1	22	—	—	3	1				
		5	5										1	1	1	11	1	22	—	—	3	1				
		6											4	1	1	11	1	22	—							
	7	1	1										1	1	1	11	1	22	—	—	3	1				
		2	2										4	1	1	11	1	21	—	—	2	1				
		3	3										4	1	1	11	1	22	—	—	4	1				
		4	4										5	1	1	10	1	22	—	—	2	1				
		5	5										1	1	1	11	1	22	—	—	2	1				
		6											4	1	1	11	1	22	—							
	10	1	1										~~4~~	1	1	11	1	22	—	—	4	1				
		2	2										~~1~~	1	1	11	1	22	—	—	4	1				
		3	3										4	1	1	11	1	22	—	—	4	1				
		4	4										5	1	1	10	1	22	—	—	5	1				
		5	5										1	1	1	11	1	22	—	—	4	1				
		6											1	1	1	11	1	22	—							
6	4	1	1										1	1	1	11	1	22	—	—	5	1				
		2	2										4	1	1	11	1	22	—	—	3	1				
		3	-										4	1	1	11	1	22	—		3	1				
		4	4										9	1	1	10	1	22	—	—	3	1				
		5	5										2	1	1	11	1	22	—	—	3	1				
		6											4	1	1	11	1	22	—							
	7	1	1										1	1	1	11	1	22	—	—	2	1				
		2	2										4	1	1	11	1	22	—	—	5	1				
		3	3										4	1	1	11	1	22	—		2	1				
		4	4										9	1	1	10	1	22	—		2	1				
		5	5										2	1	1	11	1	22	—		2	1				
		6											4	1	1	11	1	22	—							
	10	1	1										1	1	1	11	1	22	—		4	1				
		2	2										4	1	1	11	1	22	—	—	2	1				
		3	3										1	1	1	11	1	22	—	—	4	1				
		4	4										9	1	1	10	1	22	—	—	5	1				
		5	5										2	1	1	11	1	22	—	—	4	1				
		6											1	1	1	11	1	22	—							

Table 10

NAME	INVESTIGATOR. Day of observation	Agar No	Gelatin No	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Form in Rod.	Spores.	Germination.	Capsule.	Stain by Gram.	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	Liquefaction
cillus coli 7	4	1	1											1	1	1	11	1	22	–	–	3	1				
		2	2											4	1	1	11	1	22	–	–	3	1				
		3	3											4	1	1	11	1	22	–	–	3	1				
		4	4											1	1	1	10	1	22	–	–	3	1				
		5	5											2	1	1	11	1	22	–	–	3	1				
		6												4	1	1	11	1	22	–							
	7	1	1											1	1	1	11	1	22	–	–	2	1				
		2	2											4	1	1	11	1	22	–	–	2	1				
		3	3											4	1	1	11	1	22	–	–	2	1				
		4	4											1	1	1	10	1	22	–		2	1				
		5	5											2	1	1	11	1	22	–	–	2	1				
		6												4	1	1	11	1	22	–							
	10		1											1	1	1	11	1	22	–	–	2	1				
		2	2											4	1	1	11	1	22	–	–	2	1				
		3	3											4	1	1	11	1	22	–	–	2	1				
		4	4											1	1	1	10	1	22	–	–	4	1				
		5	5											2	1	1	11	1	22	–	–	2	1				
		6												4	1	1	11	1	22	–							
8	4	1	1											1	1	1	11	1	22	–	–	3	1				
		2	2											4	1	1	11	1	22	–		3	1				
		3	3											4	1	1	11	1	22	–		3	1				
		4	4											4	1	1	10	1	22	–		3	1				
		5	5											2	1	1	11	1	22	–	–	3	1				
		6												1	1	1	11	1	22	–							
	7	1	1											1	1	1	11	1	22	–	–	2	1				
		2	2											4	1	1	11	1	22	–	–	5	1				
		3	3											4	1	1	11	1	22	–	–	2	1				
		4	4											4	1	1	10	1	22	–		3	1				
		5	5											2	1	1	11	1	22	–	–	5	1				
		6												1	1	1	11	1	22	–							
	10	1	1											1	1	1	11	1	22	–	–	4	1				
		2	2											4	1	1	11	1	22	–	–	5	1				
		3	3											4	1	1	11	1	22	–	–	[illegible]	1				
		4	4											4	1	1	10	1	22	–	–	[illegible]	1				
		5	5											2	1	1	11	1	22	–	–	5	1				
		6												4	1	1	11	1	22	–							

NAME.	INVESTIGATOR.	Day of observation	Agar No.	Gelatin No.	TYPE NUMBER.	Diameter + 1 micron.	Form in Rod.	Germination.	Spores.	Capsule.	Stain by Gram.	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	S. Liquefaction.
illus coli	9	4	1	1											1	1	1	11	1	22	–	5	–	+				
			2	2											1	1	1	11	1	22	–	5		+				
			3	3											1	1	1	11	1	22	–	5	–	+				
			4	4											4	1	1	10	1	22	–	5	–	+				
			5	5											1	1	1	11	1	22	–	5	–	+				
			6												1	1	1	11	1	22	–							
		7	1	1											1	1	1	11	1	22	–	5		+				
			2	2											1	1	1	11	1	22	–	5	–	+				
			3	3											1	1	1	11	1	22	–	5	–	+				
			4	4											4	1	1	10	1	22	–	5		+				
			5	5											1	1	1	11	1	22	–	5	–	+				
			6												1	1	1	11	1	22	–							
		10	1	1											1	1	1	11	1	22	–	5	–	+				
			2	2											1	1	1	11	1	22	–	5		+				
			3	3											1	1	1	11	1	22	–	5	–	+				
			4	4											4	1	1	10	1	22	–	5	–	+				
			5	5											1	1	1	11	1	22	–	5	–	+				
			6												1	1	1	11	1	22	–							
	10	4	1	1											1	1	1	11	1	22	–	–	3	1				
			2	2											1	1	1	11	1	22	–	–	3	1				
			3	3											3	1	1	11	1	22	–	–	3	1				
			4	4											1	1	1	10	1	22	–	–	3	1				
			5	5											1	1	1	11	1	22	–	–	3	1				
			6												1	1	1	11	1	22	–							
		7	1	1											1	1	1	11	1	22	–	–	3	1				
			2	2											1	1	1	11	1	22	–	–	3	1				
			3	5											3	1	1	11	1	22	–	–	3	1				
			4	4											1	1	1	10	1	22	–	–	4	1				
			5	5											1	1	1	11	1	22	–	–	2	1				
			6												1	1	1	11	1	22	–							
		10	1	1											1	1	1	11	1	22	–	–	2	1				
			2	2											1	1	1	11	1	22	–	–	2	1				
			3	5											4	1	1	11	1	22	–	–	4	1				
			4	4											1	1	1	10	1	22	–	–	2	1				
			5	5											4	1	1	11	1	22	–	–	2	1				
			6												1	1	1	11	1	22	–							

Table 12

NAME.	INVESTIGATOR. Day of observation	Agar No.	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.
[Ba]cillus typhosus 1	10	1	1										1	1	1	11	1	22	–	–	2	1			
		2	2										4	1	1	11	1	22	–	–	2	1			
		3	3										4	1	1	11	1	22	–	–	2	1			
		4	4										4	1	1	11	1	22	–	–	2	1			
		5	5										3	1	1	11	1	22	–	–	2	1			
		6											4	2	1	11	1	22	–						
2	10	1											1	1	1	11	1	22	–	–	2	1			
		2	2										4	1	1	11	1	22	–	–	2	1			
		3	3										1	1	1	11	1	22	–	–	2	1			
		4	4										1	1	1	11	1	22	–	–	2	1			
		5	5										2	1	1	11	1	22	–	–	2	1			
		6											1	1	1	11	1	22	–						
3	10	1	1											1	1	11	1	22	–	–	2	1			
		2	2										1	1	1	11	1	22	–		2	1			
		3	3										1	1	1	11	1	22	–	–	2	1			
		4	4										1	1	1	11	1	22	–	–	2	1			
		5	5										1	1	1	11	1	22	–	–	2	1			
		6											1	1	1	11	1	22	–						
4	10	1	1										1	1	1	11	1	22	–	–	2	1			
		2	2										1	1	1	11	1	22	–	–	2	1			
		3	3										1	1	1	11	1	22	–	–	2	1			
		4	4										1	1	1	11	1	22	–	–	2	1			
		5	5										1	1	1	11	1	22	–		4	1			
		6											3	1	1	11	1	22	–						
5	10	1	1										4	1	1	11	1	22	–	–	5	1			
		2	2										1	1	1	11	1	22	–	–	5	1			
		3	3										1	1	1	11	1	22	–	–	5	1			
		4	4										1	1	1	11	1	22	–	–	5	1			
		5	5										3	1	1	11	1	22	–	–	5	1			
		6											1	1	1	11	1	22	–						
6	10	1	1										2	1	1	11	1	22	–	–	2	1			
		2	2										1	1	1	11	1	22	–		2	1			
		3	3										1	1	1	11	1	22	–	–	2	1			
		4	4										4	1	1	11	1	22	–	–	2	1			
		5	5										2	1	1	11	1	22	–	–	2	1			
		6											1	1	1	11	1	22	–						

Table 13

NAME.	INVESTIGATOR. Day of observation	Agar No.	Gelatin No	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Form in Rod. Spores.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	Liquefaction.
...illus typhosus 7	10	1	1										4	1	1	11	1	22	—	—	2	1				
		2	2										1	1	1	11	1	22	—	—	2	1				
		3	3										4	1	1	11	1	22	—	—	2	1				
		4	4										1	1	1	11	1	22	—	—	2	1				
		5	5										2	1	1	11	1	22	—	—	2	1				
		6											3	1	1	11	1	22	—							
8	10	1	1										1	1	1	11	1	22	—	—	2	1				
		2	2										3	1	1	11	1	22	—	—	2	1				
		3	3										4	1	1	11	1	22	—	—	2	1				
		4	4										1	1	1	11	1	22	—	—	2	1				
		5	5										1	1	1	11	1	22	—	—	2	1				
		6											4	1	1	11	1	22	—							
4	10	1	1										1	1	1	11	1	22	—	—	2	1				
		2	2										3	1	1	11	1	22	—		4	1				
		3	3										4	1	1	11	1	22	—	—	5	1				
		4	4										1	1	1	11	1	22	—	—	5	1				
		5	5										2	1	1	11	1	22	—	—	5	1				
		6											3	1	1	11	1	22	—							
10	10	1	1										1	1	1	11	1	22	—		4	1				
		2	2										4	1	1	11	1	22	—	—	5	1				
		3	3										4	1	1	11	1	22	—	—	2	1				
		4	4										4	1	1	11	1	22	—		2	1				
		5	5										2	1	1	11	1	22	—		2	1				
		6											3	1	1	11	1	22	—							
11	10	1	1										3	1	1	11	1	22	—	—	2	1				
		2	2										1	1	1	11	1	22	—	—	2	1				
		3	3										3	1	1	11	1	22	—	—	5	1				
		4	4										1	1	1	11	1	22	—		2	1				
		5	5										2	1	1	11	1	22	—	—	2	1				
		6											1	1	1	11	1	22	—							

Table 14

NAME.	INVESTIGATOR. Day observation	Agar No	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	S Liquefaction.
cillus typhosus 1	4	1	1										1	1	1	11	1	22	–	–	1	–				
		2	2										4	[illegible]	1	11	1	22			1	–				
		3	3										4	1	1	11	1	22		–	1	–				
		4	4										1	1	1	11	1	22	–	–	1	–				
		5	5										4	1	1	11	1	22	–	–	1	–				
		6											4	1	1	11	1	22	–							
	7	1	1										1	1	1	11	1	22	–	–	2	1				
		2	2										4	1	1	11	1	22	–	–	2	1				
		3	3										1	1	1	11	1	22	–	–	2	1				
		4	4										1	1	1	11	1	22	–	–	1	–				
		5	5										2	1	1	11	1	22	–	–	2	1				
		6											1	1	1	11	1	22	–							
	10	1	1										1	1	1	11	1	22	–	–	2	1				
		2	2										4	1	1	11	1	22	–	–	2	1				
		3	3										4	1	1	11	1	22	–	–	2	1				
		4	4										4	1	1	11	1	22	–	–	1					
		5	5										3	1	1	11	1	22	–	–	2	1				
		6											4	2	1	11	1	22	–							
2	4	1	1										1	1	1	11	1	22	–	–	1	–				
		2	2										1	1	1	11	1	22	–	–	1	–				
		3	3										1	1	1	11	1	22	–	–	1	–				
		4	4										1	1	1	11	1	22	–	–	1	–				
		5	5										2	1	1	11	1	22	–	–	2	–				
		6											1	1	1	11	1	22	–							
	7	1	1										1	1	1	11	1	22	–	–	2	1				
		2	2										1	1	1	11	1	22	–	–	2	1				
		3	3										1	1	1	11	1	22	–	–	2	1				
		4	4										1	1	1	11	1	22	–	–	2	1				
		5	5										7	1	1	11	1	22	–	–	2	1				
		6											4	1	1	11	1	22	–							
	10	1	1										1	1	1	11	1	22	–	–	2	1				
		2	2										4	1	1	11	1	22	–	–	2	1				
		3	3										1	1	1	11	1	22	–	–	2	1				
		4	4										1	1	1	11	1	22	–	–	2	1				
		5	5										2	1	1	11	1	22	–	–	2	1				
		6											1	1	1	11	1	22	–							

Table 15

NAME.	INVESTIGATOR. Day observation	Agar No	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	S Liquefaction.
illus typhosus 3	4	1	1										1	1	1	11	1	22	-	-	1	-				
		2	2										1	1	1	11	1	22	-	-	3	+				
		3	3										4	1	1	11	1	22	-	-	1	-				
		4	4										1	1	1	11	1	22	-	-	1	-				
		5	5										2	1	1	11	1	22	-	-	1	-				
		6											4			11	1	22	-							
	7	1	1										1	1	1	11	1	22	-	-	3	-				
		2	2										1		1	11	1	22	-	-	3	-				
		3	3										1	1	1	11	1	22	-	-	3	-				
		4	4										1	1	1	11	1	22	-	-	3	-				
		5	5										1	1	1	11	1	22	-	-	3	-				
		6											4	1	1	11	1	22	-							
	10	1	1										1	1	1	11	1	22	-	-	2	1				
		2	2										1	1	1	11	1	22	-	-	2	1				
		3	3										1	1	1	11	1	22	-	-	2	1				
		4	4										1	1	1	11	1	22	-	-	2	1				
		5	5										1	1	1	11	1	22	-	-	2	1				
		6											1	1	1	1	1	22	-							
4	4	1	1										1		1	11	1	22	-	-	1	-				
		2	2										1	1	1	11	1	22	-		1	-				
		3	3										1	1	1	11	1	22	-	-	1	-				
		4	4											1	1	11	1	22	-	-	1	-				
		5	5										2	1	1	11	1	22	-	-	1	-				
		6											4	1	1	11	1	22	-							
	7	1	1										1	1	1	11	1	22	-	-	1	1				
		2	2										1	1	1	11	1	22	-	-	3	1				
		3	3										1	1	1	11	1	22	-	-	3	-				
		4	4										1		1	11	1	22	-	-	3	-				
		5	5										2	1	1	11	1	22	-	-	3	-				
		6											4	1	1	11	1	22	-							
	10	1	1										1	1	1	11	1	22	-	-	2	1				
		2	2										1	1		11	1	22	-	-	3	1				
		3	3										1	1	1	11	1	22	-	-	3	1				
		4	4										1	1	1	11	1	22	-	-	2	1				
		5	5										3	1	1	11	1	22	-	-	4	1				
		6											4	1	1	11	1	22	-							

Table 16

NAME.	INVESTIGATOR. Day observation	Agar No	Gelatin No	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	Liquefaction.
illus typhosus 5	4	1	1										4	/	/	//	/	22	—	—	/	—				
		2	2										/	/	/	//	/	22	—	—	/	—				
		3	3										4	/	/	//	/	22	—	—	/	—				
		4	4										/	/	/	//	/	22	—	.	/	—				
		5	5										/	/	/	//	/	22	—	—	/	/				
		6											4	/	/	//	/	22	—							
	7	1	1										4	/	/	//	/	22	—	—	2					
		2	2										/	/	/	//	/	22	—		2	—				
		3	3										/	/	/	//	/	22	—	-	3	—				
		4	4										/	/	/	//	/	22	—	—	2	—				
		5	5										2	/	/	//	/	22	—	—	3	—				
		6											/	/	/	//	/	22	—							
	10	1	1										4	/	/	//	/	22	—	-	5	/				
		2	2										/		/	//	/	22	—	.	5	/				
		3	3										/	/	/	//	/	22	—	—	5	/				
		4	4										/	/	/	//	/	22	—	—	5	/				
		5	5										3	/	/	//	/	22	—	-	5	/				
		6											/	/	/	//	/	22	—							
6	4	1	1										3	/	/	//	/	22	—	—	/	—				
		2	2										4	/	/	//	/	22	—	—	/	—				
		3	3										4	/	/	//	/	22	—	—	/	—				
		4	4										/	/	/	//	/	22	—	—	/	—				
		5	5+										/	/	/	//	/	22	—	—	/	—				
		6											/	/	/	//	/	22	—							
	7	1	1										3	/	/	//	/	22	—	—	3	—				
		2	2										4	/	/	//	/	22	—	—	3	—				
		3	3										4	/	/	//	/	22	—	—	3	/				
		4	4										/	/	/	//	/	22	—	—	3	—				
		5	5+										/	/	/	//	/	22	—	—	3	/				
		6											/	/	/	//	/	22	—							
	10	1	1										2	/	/	//	/	22	—	—	2	/				
		2	2										/	/	/	//	/	22	—		2	/				
		3	3										/	/	/	//	/	22	—	—	2	/				
		4	4										4	/	/	//	/	22	—	—	2	/				
		5	5+										2	/	/	//	/	22	—		2	/				
		6											/	/	/	//	/	22	—							

Table 17

NAME.	Day observation	Agar No.	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram.	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	AGAR. Elevation of Growth.	AGAR. Lustre.	AGAR. Chromogenesis.	AGAR. Topography of Growth.	AGAR. Optical Characters.	AGAR. Viscosity.	GEL. Liquefaction—Form.	GEL. Line Growth.	GEL. Surface Growth.	BROTH. Pellicle.	BROTH. Turbidity.	BROTH. Sediment.	Liquefaction.
llus typhosus 7	4	1	1										4	1	1	11	1	22	-	—	1	-				
		2	2										1	1	1	11	1	22	—	—	1	—				
		3	3										1	1	1	11	1	22	—	—	1	—				
		4	4										1	1	1	11	1	22	—	-	1	—				
		5	5										2	1	1	11	1	22	—	—	1	—				
		6											1	1	1	11	1	22	—							
	7	1	1										4	1	1	11	1	22	—	—	1	—				
		2	2										1	1	1	11	1	22	—	—	1	—				
		3	3										4	1	1	11	1	22	—	-	2	1				
		4	4										1	1	1	11	1	22	—	—	2	1				
		5	5										2	1	1	11	1	22	—	—	2	1				
		6											1	1	1	11	1	22	—							
	10	1	1										4	1	1	11	1	22	—	-	2	1				
		2	2										1	1	1	11	1	22	—	-	2	1				
		3	3										4	1	1	11	1	22	—	—	2	1				
		4	4										1	1	1	11	1	22	—	—	2	1				
		5	5										2	1	1	11	1	22	—	—	2	1				
		6											3	1	1	11	1	22	—							
8	4	1	1										1	1	1	11	1	22	—	—	1					
		2	2											1	1	11	1	22	—		1	—				
		3	3										4	1	1	11	1	22	—	-	1	—				
		4	4										1	1	1	11	1	22	—	—	1	—				
		5	5										2	1	1	11	1	22	—	—	1	—				
		6											4	1	1	11	1	22	—							
	7	1	1										1	1	1	11	1	22	—	—	2	1				
		2	2										1	1	1	11	1	22	—	—	2	1				
		3	3										1	1	1	11	1	22	—	—	2	1				
		4	4										1	1	1	11	1	22	-	-	2					
		5	5										1	1	1	11	1	22	-	—	2	1				
		6											4	1	1	11	1	22	—							
	10	1	1										1	1	1	11	1	22	-	-	2	1				
		2	2										3	1	1	11	1	22	—	—	2					
		3	3										4	1	1	11	1	22	—	-	2	1				
		4	4										1	1	1	11	1	22	—	-	2	1				
		5	5										1	1	1	11	1	22	—	-	2	1				
		6											4	1	1	11	1	22	-							

NAME.	INVESTIGATOR. Day observation	Agar No.	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	AGAR. Elevation of Growth.	AGAR. Lustre.	AGAR. Chromogenesis.	AGAR. Topography of Growth.	AGAR. Optical Characters.	AGAR. Viscosity.	GEL. Liquefaction—Form.	GEL. Line Growth.	GEL. Surface Growth.	BROTH. Pellicle.	BROTH. Turbidity.	BROTH. Sediment.	Liquefaction.
illus typhosus 9	4	1	1										1	1	1	11	1	22	—	—	1	—				
		2	2										1	1	1	11	1	22	—	—	1	—				
		3	3										4	1	1	11	1	22	—	—	1	—				
		4	4										1	1	1	11	1	22	—	—	1	—				
		5	5										2	1	1	11	1	22	—	—	1	—				
		6											1	1	1	11	1	22	—							
	7	1	1										1	1	1	11	1	22	—	—	2	1				
		2	2										1	1	1	11	1	22	—	—	2	1				
		3	3										4	1	1	11	1	22	—	—	2	—				
		4	4										1	1	1	11	1	22	—	—	2	—				
		5	5										2	1	1	11	1	22	—	—	2	1				
		6											1	1	1	11	1	22	—							
	10	1	1										1	1	1	11	1	22	—	—	2	1				
		2	2										3	1	1	11	1	22	—	—	4	1				
		3	3										4	1	1	11	1	22	—	—	5	1				
		4	4										1	1	1	11	1	22	—	—	5					
		5	5										2	1	1	11	1	22	—	—	5	1				
		6											3	1	1	11	1	22	—							
10	4	1	1										4	1	1	11	1	22	—	—	1	—				
		2	2										1	1	1	11	1	22	—	—	1	—				
		3	3										4	1	1	11	1	22	—	—	1	—				
		4	4										1	1	1	11	1	22	—	—	1	—				
		5	5										2	1	1	11	1	22	—	—	1	1				
		6											4	1	1	11	1	22	—							
	7	1	1										4	1	1	11	1	22	—	—	3	1				
		2	2										4	1	1	11	1	22	—	—	3	1				
		3	3										4	1	1	11	1	22	—	—	3	1				
		4	4										4	1	1	11	1	22	—	—	3	1				
		5	5										2	1	1	11	1	22			3	1				
		6											4	1	1	11	1	22	—							
	10	1	1										4	1	1	11	1	22	—	—	4	1				
		2	2										4	1	1	11	1	22	—	—	5	1				
		3	3										4	1	1	11	1	22	—	—	2	1				
		4	4										2	1	1	11	1	22	—	—	2	1				
		5	5										3	1	1	11	1	22	—	—	2	1				
		6											3	1	1	11	1	22	—							

Table 19

NAME	INVESTIGATOR. Day observation	Agar No.	Gelatin No.	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram.	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	S… Liquefaction.
llus typhosus 11	4	1	1										4	·	1	11	1	22	—	—	1	—				
		2	2										1	1	1	11	1	22	—		1	—				
		3	3										4	1	1	11	1	22	—	·	1	—				
		4	4										4	1	1	11	1	22	—	--	1	—				
		5	5										2	1	1	11	1	22	—	·	1	—				
		6											1	1	1	11	1	22	—							
	7	1	1										4	1	1	11	1	22	—	—	3	1				
		2	2										1	1	1	11	1	22	—	—	3	1				
		3	3										4	1	1	11	1	22	—	—	3	1				
		4	4										4	1	1	11	1	22	—	—	3	1				
		5	5										2	1	1	11	1	22	—	—	3	1				
		6											1	1	1	11	1	22	—							
	10	1	1										3	1	1	11	1	22	—	-	2	1				
		2	2										1	1	1	11	1	22	—	—	2	1				
		3	3										3	1	1	11	1	22	—	-	5	1				
		4	4										1	1	1	11	1	22	—	—	2	1				
		5	5										2	1	1	11	1	22	—	—	2	1				
		6											1	1	1	11	1	22	-							

Table 20

NAME.	INVESTIGATOR. Day observation	Agar No.	Gelatin No	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	S[…] Liquefaction.
us prodigiosus 1.	4	1	1										1	4	x4	30	1	30	+	3	−	+				
		2	2										1	3	1	x32	1	30	+	2	−	+				
		3	3										1	3	1	x32	1	30	+	2	−	+				
		4	5										9	4	1	x32	1	30	+	2	−	+				
		7	6										4	1	1	32	7	30	+	2	−	+				
		8											4	1	1	30	1	30	+							
	7	1	1										1	4	+4	30	1	30	+	3	−	−				
		2	2										1	4	1	x32	1	30	+	3	−	−				
		3	3										1	4	1	x32	1	30	+	3	−	−				
		4	5										9	1	1	x32	1	30	+	3	−	−				
		7	6										4	4	1	x32	1	30	+	3	−	−				
		8											4	4	1	30	1	30	+							
	10	1	1										1	4	+4	30	1	30	+	3	−	−				
		2	2										1	4	1	x32	1	30	+	3	−	−				
		3	3										1	4	1	x32	1	30	+	3	−	−				
		4	5										9	4	1	x32	1	30	+	3	−	−				
		7	6										4	4	1	x32	1	30	+	3	−	−				
		8											4	4	1	30	1	30	+							
2	4	1	1										3	4	1	38	1	30	+	4	−	+				
		2	2										1	3	+4	32	1	30	+	5	−	+				
		3	3										1	3	+4	32	1	30	+	5	−	+				
		4	5										4	3	1	x32	1	30	+	5	−	+				
		7	6										3	3	1	32	1	30	+	5	−	+				
		8											2	3	1	38	1	30	+							
	7	1	1										3	4	1	30	1	30	+	3	−	+				
		2	2										1	4	1	32	1	30	+	5	−	+				
		3	3										1	4	1	32	1	30	+	5	−	+				
		4	5										9	4		32	1	30	+	5	−	+				
		7	6										2	4	1	32	1	30	+	5	−	+				
		8											4	4	2	30	1	30	+							
	10	1	1										3	4	1	30	1	30	+	3	−	+				
		2	2										1	4	1	32	1	30	+	5	−	+				
		3	3										1	4	1	32	1	30	+	5	−	+				
		4	5										9	4	1	32	1	30	+	5		+				
		7	6										2	4	1	32	1	30	+	5		+				
		8											4	4	2	30	1	30	+							

+ lustre like fuchsin.
x condensation water scarlet

Table 21

NAME	INVESTIGATOR.	Day observation	Agar No	Gelatin No	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Form in Rod. Spores.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	Liquefaction
...us prodigiosus	3	4	1	1										1	3	1	38	1	30	–	3	–	–				
			2	2										1	3	1	32+	1	30	–	5	–					
			3	3										1	3	1	32+	1	30	+	3		+				
			4	5										9	1	1	33	1	30	+	3		-				
			7	6										1	3	1	30	1	30	–	3	–	–				
			8											1	3	1	38	1	30	–							
		7	1	1										1	4	1	38		30	–	3	–	+				
			2	2										1	4	1	32+	1	30	–	5	–	+				
			3	3										1	4	1	32+	1	30	+	3		+				
			4	5										7	1	1	33		30	+	3		+				
			7	6										1	3	1	32	1	30	–	3		+				
			8											1	3	1	38	1	30	–							
		10	1	1										1	4	1	38	1	30	–	3	–	+				
			2	2										1	4	1	32+	1	30	–	5	–	+				
			3	3										1	4	1	32+	1	30	+	5	–	+				
			4	5										4	1	1	33	1	3	+	3	–	+				
			1	6										1	3	1	32		3[illegible]	–	5						
			8											1	3	1	38	1	3[illegible]	–							
	4	4	1	1										1	4	1	38	1	30	–	3	–	–				
			2	2										1	4	1	32+	1	30		3	–					
			3	3										1	4	1	32+	1	30	–	3		–				
			4	5										7	4	1	33+	1	30	–	3		–				
			7	6										1	4	1	32	1	30	–	3	–	–				
			8											1	4		38	1	30	–							
		7	1	1										1	4	1	38	1	30		3	–	–				
			2	2										1	4	1	32+	1	30	–	3	–	–				
			3	3										1	4	1	32+	1	30		3	–	–				
			4	5										7	4	1	33+	1	30	–	3	–	–				
			7	6										1	4	1	32	1	30	–	3	–	–				
			8											1	4	1	38	1	30	–							
		10	1	1										1	4	1	38	1	30	–	3	–	–				
			2	2										1	4	1	32+	1	30	–	3	–	–				
			3	3										1	4	1	32+	1	30	–	3	–	–				
			4	5										7	4	1	33+	1	30		3		–				
			7	6										1	4	1	32	1	30		3	–	–				
			8											1	4	1	38	1	30								

+ Condensation water scarlet.

Table 22

NAME	Day observation	Agar No	Gelatin No	TYPE NUMBER	Diameter + 1 micron	Spores. Form in Rod.	Spores. Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	AGAR. Elevation of Growth.	AGAR. Lustre.	AGAR. Chromogenesis.	AGAR. Topography of Growth.	AGAR. Optical Characters.	AGAR. Viscosity.	GEL. Liquefaction—Form.	GEL. Line Growth.	GEL. Surface Growth.	BROTH. Pellicle.	BROTH. Turbidity.	BROTH. Sediment.	S… Liquefaction.
…us prodigiosus 5	4	1	1										1	4	1	38	1	30	–	3	–	+				
		2	2										1	4	1	38	1	30	–	3	–	–				
		3	3										1	4	1	38	1	30	–	3	–	–				
		4	5										1	1	1	38		30	–	3	–	–				
		7	6										1	1	1	38	1	30	–	3	–	–				
		8											1	1	1	38	1	30	–							
	7	1	1										1	3	1	38	1	30	–	3	–	+				
		2	2										1	3	1	38	1	30	–	3	–	+				
		3	3											3		38		30	–	3	–	–				
		4	5										1	1	1	38		30	–	3	–	–				
		7	6										1	3	1	38	1	30	–	3	–	–				
		8											1	3	1	38	1	30	–							
	10	1	1										1	3	1	38	1	30	–	3	–	+				
		2	2											3	1	38	1	30	–	3	–	–				
		3	3											3	1	38	1	30	–	3	–	–				
		4	5										1	1	1	38	1	30	–	3	–	–				
		7	6										1	3	1	38	1	30	–	3	–	–				
		8											1	3	1	38	1	30	–							
6	4	1											1	3	1	38	1	30	–	3	–	–				
		2	2										1	4	1	32	1	30	–	3	–	–				
		3	3										1	6	1	32	1	30	–	5	–	–				
		4	5										4	4	1	38	1	30	–	5	–	–				
		7	6										1	4	1	38	1	30	–	3	–	–				
		8											1	4	1	38	1	30	–							
	7	1	1										1	3	1	38	1	30	–	3	–	–				
		2	2										1	4	1	32+	1	30	–	3	–	–				
		3	3										1	6		32+	1	30	–	5		–				
		4	5										4	1	1	38	1	30	–	5	–	–				
		7	6										1	4	1	38		30	–	3	–	–				
		8											2	4	1	38	1	30	–							
	10	1	1										1	3	1	38	1	30	–	3	–	–				
		2	2										1	4	1	32+	1	30	–	3	–	–				
		3	3										1	6	1	32+	1	30	–	5	–	–				
		4	5										4	1	1	38	1	30	–	5	–	–				
		7	6										1	4	1	38	1	30	–	3	–					
		8											2	4	1	38	1	30	–							

+. Condensation water scarlet.

Table 23

NAME.	INVESTIGATOR.			TYPE NUMBER.	MORPHOLOGY.									AGAR.							GEL.			BROTH.			S
	Day observation	Agar No.	Gelatin No.		Diameter + 1 micron.	Form in Rod.	Spores.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	Liquefaction—Form.	Line Growth.	Surface Growth.	Pellicle.	Turbidity.	Sediment.	Liquefaction
lus prodigiosus 7.	4	1	1											1	3		38	1	30	-	3	-	-				
		2	2											4	4	1	32	1	30	-	3	-	-				
		3	3											4	6	1	32	1	30	-	3	-	-				
		4	5											9	1	1	33	1	30	-	3	-	-				
		7	6											1	4	1	38	1	30	-	5	-	-				
		8												1	4		38	1	30	-							
	7	1	1											1	3	1	38	1	30	+	3	-	-				
		2	2											1	4	1	+32	1	30	+	3	-	-				
		3	3												6	1	+32		30	+	3	-	-				
		4	5											9	1	1	+33	1	30	-	3	-	-				
		7	6											1	6	1	32	1	30	-	5	-	-				
		8												1	4	1	38	1	30	-							
	10	1	1											1	3	1	38	1	30	+	3	-	-				
		2	2											1	4	1	+32	1	30	+	3	-	-				
		3	3											1	6	1	+32	1	30	+	3	-	-				
		4	5											9	1	1	+33	1	30	-	3	-	-				
		7	6											1	6		+32	1	30	-	5	-	-				
		8												1	4	1	38	1	30	-							
8	4	1	1											1	4	1	38	1	30	-							
		2	2											1	3	1	32	1	30	-							
		3	3											1	3	1	32	1	30	-							
		4	5											9	1		33	1	30	-							
		7	6											1	3		38	1	30	-							
		8												1	3	1	38	1	30	-							
	7	1	1											1	4	1	38	1	30	-							
		2	2											1	4	1	+32	1	30	+							
		3	3											1	4	1	+32	1	30	+							
		4	5											9	1	1	+33	1	30	-							
		7	6											1	4	4	+32	1	30	-							
		8													3	1	38	1	30	-							
		1	1											1	4	1	38	1	30	-							
		2	2											1	4	1	+32	1	30	+							
		3	3											1	4	1	+32	1	30	+							
		4	5											9	1	1	+33	1	30	-							
		7	6											1	4	4	+32	1	30	-							
		8												1	3	1	38	1	30	-							

Table 21

NAME.	INVESTIGATOR. Day observation	Agar No	Gelatin No	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Spores.	Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment	Liquefaction
cillus indicus	4	1	1											9	1	1	+30	1	30	·	3	–	+				
		2	2											9	1	1	30	1	30	–	3	–	+				
		3	3											1	1	1	30	1	30	–	3	–	+				
		4	5											9	1	1	11	1	30	–	3	–	+				
		6	6											9	1	1	32	1	30	–	3	–	+				
		7												9	1	1	38	1	30	–							
		8												9	1	1	38	1	30	–							
	7	1	1											9	1	1	38	1	30	–	3		+				
		2	2											9	1	1	32	1	30	–	3	–	+				
		3	3											1	1	1	38	1	30	–	3	–	+				
		4	5											9	1	1	22	1	30	–	3	–	+				
		6	6											9	1	1	32	1	30	–	3		+				
		7												9	1	1	+32	1	30	–							
		8												5	1	1	38	1	30	–							
	10	1	1											7	1	1	38	1	30	–	3	–	+				
		2	2											4	1	1	32	1	30	–	3	–	+				
		3	3											1	1	1	38	1	30	–	3	–	+				
		4	5											9	1	1	22	1	30	–	3	–	+				
		6	6											9	1	1	+32	1	30	–	3		+				
		7												9	1	1	+32	1	30	–							
		8												5	1	1	38	1	30	–							
														x													

x medium dissolved in every case.
+ condensation water

Table 25

NAME.	Day observation	Agar No.	TYPE NUMBER.	Fluorescence	Diameter + 1 micron.	Spores.	Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	AGAR. Elevation of Growth.	AGAR. Lustre.	AGAR. Chromogenesis.	AGAR. Topography of Growth.	AGAR. Optical Characters.	AGAR. Viscosity.	GEL. Liquefaction—Form.	GEL. Line Growth.	GEL. Surface Growth.	BROTH. Pellicle.	BROTH. Turbidity.	BROTH. Sediment.	SE… Liquefaction
us pyocyaneus. 1	10	1		92										4	1	1	11	1	30	-							
		2		92										1	1	1	11	1	30	-							
		3		96										1	1	1	11	1	30	-							
		4		95										1	1	1	11	1	30	-							
		7		96										1	1	1	11	1	30	-							
		8		96										4	1	1	11	1	30	-							
		10		96										1	1	1	11	1	30	-							
		11		96										1	1	1	11	1	30	-							
		12		96										1	1	1	11	1	30	-							
		13		-										1	1	1	11	1	30	-							
		14		93										1	1	1	11	1	30	-							
2	10	1		96										1	1	1	11	1	30	-							
		2		96										1	1	1	11	1	30	-							
		3		96										1	1	1	11	1	30	-							
		4		92										1	1	1	24	1	30	-							
		7		97										4	1	2	11	1	30	-							
		8		97										4	1	1	11	1	30	-							
		10		92										1	1	1	21	1	30	-							
		11		96										1	1	2	24	1	30	-							
		12		96										1	1	4	11	1	30	-							
		13		95										1	1	4	11	1	30	-							
		14		96										1	1	4	11	1	30	-							
3	10	1		96										5	1	1	11	1	30	-							
		2		-										1	1	1	22	1	30	-							
		3		-										1	1	1	22	1	30	-							
		4		95										1	1	1	11	1	30	-							
		7		97										1	1	1	11	1	30	-							
		8		-										1	4	1	26	1	30	-							
		10		96										1	1	1	11	7	30	-							
		11		92										1	1	1	22	1	30	-							
		12		92										1	1	1	11	1	30	-							
		13		95										1	1	1	11	1	30	-							
		14		96										1	1	1	11	1	30	-							

Table 26

NAME	Day observation	INVESTIGATOR	Agar No.	TYPE NUMBER	Fluorescence	MORPHOLOGY. Diameter + 1 micron	Form in Rod. Spores.	Germination.	Capsule.	Stain by Gram.	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	S… Liquefaction
…llus pyocyaneus	4	10	1		96									3	1	4	11	1	30	–							
			2		–									4	1	4	34	5	30	–							
			3		96									4	1	4	64	5	30	–							
			4		95									4	1	4	23	5	30	–							
			7		95									1	1	1	63	1	30	–							
			8		95									1	4	1	11	7	30	–							
			10		96									1	1	1	63	1	30	–							
			11		–									4	1	4	11	5	30	–							
			12		92									1	1	1	11	5	30	–							
			13		92									1	1	1	21	1	30	–							
			14		92									1	1	4	24	1	30	–							
	5	10	1		96									1	1	1	61	1	30	–							
			2		–									1	1	1	34	1	30	–							
			3		96									1	1	1	62	1	30	–							
			4		–									4	1	1	11	1	30	–							
			7		96									1	1	1	11	1	30	–							
			8		96									1	1	1	11	1	30	–							
			10		–									1	1	1	75	1	30	–							
			11		96									4	1	1	61	1	30	–							
			12		95									1	1	1	62	1	30	–							
			13		–									1	1	1	11	1	30	–							
			14		96									1	1	1	62	1	30	–							
	6	10	1		96									1	3	1	65	5	30	–							
			2		96									1	1	1	31	1	30	–							
			3		96									1	1	1	11	1	30	–							
			4		96									1	1	1	11	1	30	–							
			7		92									1	1	1	11	1	30	–							
			8		92									1	1	2	11	1	30	–							
			10		97									1	1	4	11	1	30	–							
			11		97									1	1	4	11	1	30	–							
			12		92									1	1	4	24	1	30	–							
			13		95									1	1	1	57	1	30	–							
			14		96									1	1	4	61	5	30	–							

Table 27

NAME.	INVESTIGATOR. Day observation	Agar Number	TYPE NUMBER.	Fluorescence	MORPHOLOGY. Diameter + 1 micron.	Spores. Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity	Sediment.	SE Liquefaction
s pyocyaneus	7/10	1		94									1	1	1	11	1	30	–							
		2		–									1	1	1	11	1	30	–							
		3		–									3	1	1	11	1	30	–							
		4		96									1	1	1	11	1	30	–							
		7		96									1	1	1	11	1	30	–							
		8		96									1	3	1	11	1	30	–							
		10		96									1	1	1	11	1	30	–							
		11		96									1	1	1	11	1	30	–							
		12		92									1	1	1	11	1	30	–							
		13		96									1	1	1	11	1	30	–							
		14		92									1	1	1	51	1	30	–							
	8/10	1		92									4	1	1	11	1	30	–							
		2		–									1	1	1	11	1	30	–							
		3		–									1	1	1	11	1	30	–							
		4		96									1	1	1	11	1	30	–							
		7		–									1	1	1	11	1	30	–							
		8		96									1	4	4	11	1	30	–							
		10		96									1	1	1	11	1	30	–							
		11		92									1	1	1	11	1	30	–							
		12		–									1	1	1	11	1	30	–							
		13		95									1	1	1	11	1	30	–							
		14		–									1	1	1	11	1	30	–							
	9/10	1		96									1	1	1	11	5	30	–							
		2		93									1	1	4	11	5	30	–							
		3		96									1	1	4	11	5	30	–							
		4		92									1	1	4	11	5	30	–							
		7		92									1	1	1	11	1	30	–							
		8		92									1	3	4	11	5	30	–							
		10		97									1	1	4	11	5	30	–							
		11		96									1	3	4	11	5	30	–							
		12		96									1	3	4	11	5	30	–							
		13		95									1	3	4	11	5	30	–							
		14		95									1	3	4	11	5	30	–							

Table 28

NAME.	Day observation	INVESTIGATOR.	Agar No.	TYPE NUMBER.	Fluorescence	Diameter + 1 micron.	Form in Rod.	Spores.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	Liquefaction—Form.	Line Growth.	Surface Growth.	Pellicle.	Turbidity.	Sediment.	Liquefaction.
						MORPHOLOGY.									AGAR.							GEL.			BROTH.			SE
...lus pyocyaneus	10	10	1		96										1	1	1	11	1	30	–							
			2		–										1	1	1	11	1	30	–							
			3		–										5	1	1	11	1	30	–							
			4		95										1	1	1	11	1	30	–							
			7		96										1	1	1	11	1	30	–							
			8		96										1	4	1	11	1	30	–							
			10		96										1	1	1	11	1	30	–							
			11		96										1	1	1	11	1	30	–							
			12		96										1	1	1	11	1	30	–							
			13		95										1	1	1	11	1	30	–							
			14		96										1	1	1	11	1	30	–							
	11	10	1		92										1	1	1	11	1	30	–							
			2		–										1	1	1	11	1	30	–							
			3		–										1	1	1	11	1	30	–							
			4		95										1	1	1	11	1	30	–							
			7		–										1	1	1	11	1	30	–							
			8		–										1	1	1	11	1	30	–							
			10		–										1	1	1	11	1	30	–							
			11		92										1	1	1	11	1	30	–							
			12		92										1	1	1	11	1	30	–							
			13		95										1	1	1	11	1	30	–							
			14		92										1	1	1	11	1	30	–							
	12	10	1		96										1	1	1	11	1	30	–							
			2		93										1	1	1	11	1	30	–							
			3		93										1	1	1	11	1	30	–							
			4		95										1	1	1	11	1	30	–							
			7		92										1	1	1	11	1	30	–							
			8		96										1	1	1	11	1	30	–							
			10		97										1	1	1	11	1	30	–							
			11		96										1	1	1	11	1	30	–							
			12		92										1	1	1	11	1	30	–							
			13		95										1	1	1	11	1	30	–							
			14		92										1	1	1	11	1	30	–							

Table 21

NAME.	Day observation	INVESTIGATOR.	Agar No.	TYPE NUMBER. Fluorescence	MORPHOLOGY.								AGAR.							GEL.			BROTH.			S
					Diameter + 1 micron.	Form in Rod. Spores.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	Liquefaction—Form.	Line Growth.	Surface Growth.	Pellicle.	Turbidity.	Sediment.	Liquefaction
...llus prodigiosus	13	10	1	98									2	1	1	11	1	30	−							
			2	93									1	1	1	11	1	30	−							
			3	92									5	1	1	11	1	30	−							
			4	95									1	1	1	11	1	30	−							
			7	97									1	1	1	11	1	30	−							
			8	97									1	1	1	11	1	30	−							
			10	96									1	1	1	11	1	30	−							
			11	96									1	1	1	11	1	30	−							
			12	97									1	1	1	11	1	30	−							
			13	95									1	1	1	11	1	30	−							
			14	93									1	1	1	11	1	30	−							
	14	10	1	92									1	1	1	11	1	30	−							
			2	92									1	1	1	11	1	30	−							
			3	95									1	1	1	11	1	30	−							
			4	95									1	1	1	11	1	30	−							
			7	92									1	1	1	11	1	30	−							
			8	92									5	1	1	11	1	30	−							
			10	96									1	1	1	11	1	30	−							
			11	93									1	1	4	11	5	30	−							
			12	92									1	1	4	11	5	30	−							
			13	95									1	1	1	11	1	30	−							
			14	95									1	1	1	11	1	30	−							
	15	10	1	95									1	1	1	11	1	30	−							
			2	92									1	1	1	11	1	30	−							
			3	92									1	1	1	11	1	30	−							
			4	95									1	1	1	11	1	30	−							
			7	96									1	1	1	11	1	30	−							
			8	96									1	1	1	11	1	30	−							
			10	97									1	1	1	11	1	30	−							
			11	97									1	1	1	11	1	30	−							
			12	97									1	1	1	11	1	30	−							
			13	95									1	1	1	11	1	30	−							
			14	92									1	1	1	11	1	30	−							

Table 30

NAME.	INVESTIGATOR. Day observation	Agar No.	TYPE NUMBER. Fluorescence	MORPHOLOGY. Diameter + 1 micron.	MORPHOLOGY. Spores.	MORPHOLOGY. Form in Rod.	MORPHOLOGY. Germination.	MORPHOLOGY. Capsule.	MORPHOLOGY. Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	AGAR. Elevation of Growth.	AGAR. Lustre.	AGAR. Chromogenesis.	AGAR. Topography of Growth.	AGAR. Optical Characters.	AGAR. Viscosity.	GEL. Liquefaction—Form.	GEL. Line Growth.	GEL. Surface Growth.	BROTH. Pellicle.	BROTH. Turbidity.	BROTH. Sediment.	S… Liquefaction.
…llus pyocyaneus 16	10	1	96										1	1	1	11	1	30	–							
		2	–										1	1	1	11	1	30	–							
		3	92										1	1	1	11	1	30	–							
		4	95										1	1	1	11	1	30	–							
		7	97										1	1	1	11	1	30	–							
		8	97										1	1	1	11	1	30	–							
		10	97										1	1	1	11	1	30	–							
		11	93										1	1	1	11	1	30	–							
		12	97										1	1	1	11	1	30	–							
		13	95										1	1	1	11	1	30	–							
		14	97										1	1	1	11	1	30	–							
17	10	1	–										1	1	4	11	5	30	–							
		2	–										1	1	4	11	5	30	–							
		3	–										1	1	4	11	5	30	–							
		4	95										1	1	1	11	1	30	–							
		7	–										1	1	4	11	1	30	–							
		8	–										1	1	4	11	1	30	–							
		10	–										1	1	1	11	1	30	–							
		11	–										1	1	4	11	1	30	–							
		12	–										1	1	4	11	1	30	–							
		13	95										1	1	4	11	1	30	–							
		14	–										1	1	4	11	1	30	–							

Table 3

NAME.	INVESTIGATOR.			TYPE NUMBER.	MORPHOLOGY.									AGAR.							GEL.			BROTH.			
udent's Agar	Agar No.	Day of observation	Reaction		Diameter + 1 micron.	Spores.	Form in Rod.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	Liquefaction—Form.	Line Growth.	Surface Growth.	Pellicle.	Turbidity.	Sediment.	Liquefaction.
cillus janthinus	1	4	+0.2											2	1	1	11	1	22	−							
		10												3	1	1	11	1	22	−							
	2	4	−0.1											1	3	1	72	1	30	+							
		10												1	3	1	72	1	30	+							
	3	4	−0.2											1	4	1	71	1	30	+							
		10												1	3	1	71	1	30	+							
	4	4	0.0											1	4	1	72	1	30	+							
		10												1	3	1	72	1	30	+							
	5	4	+0.2											1	4	1	21	1	30	+							
		10												1	3	1	32	1	30	+							
	6	4	+0.2											1	4	1	71	1	30	+							
		10												1	3	1	73	1	30	+							
	7	4	−0.2											1	4	1	72	1	30	+							
		10												1	3	1	72	1	30	+							
	8	4	+1.0											1	1	1	71	1	22	+							
		10												1	3	1	73	1	22	+							
	9	4	0.0											1	1	1	11	1	22	+							
		10												1	3	1	11 ×	1	22	+							
illus prodigiosus	1	4	+0.2											6	1	1	35	1	30	−							
		10												4	1	1	35	1	30	−							
	2	4	−0.1											2	4	1	35	1	30	−							
		10												2	4	1	35	4	30	−							
	3	4	−0.2											9	1	1	33	1	30	−							
		10												9	1	1	33	1	30	−							
	4	4	0.0											1	1	1	31	1	30	−							
		10												9	1	1	30	1	30	−							
	5	4	+0.2											1	4	1	32	1	30	−							
		10												1	4	1	32	1	30	−							
	6	4	+0.2											6	1	1	30	1	30	−							
		10												6	1	1	30	1	30	−							
	7	4	−0.2											5	1	1	35	1	30	−							
		10												5	1	1	35	1	30	−							
	8	4	+1.0											4	1	1	30	1	30	−							
		10												9	1	1	30	1	30	−							
	9	4	0.0											1	1	4	30	1	30	−							
		10												9	1	4	33	1	30	−							

× Condensation water blue.

Table 32

					MORPHOLOGY.								AGAR.							GEL.			BROTH.			S
NAME.	INVESTIGATOR. Agar No.	Day of observation	Agar reaction	TYPE NUMBER.	Diameter + 1 micron.	Form in Rod. Spores.	Germination.	Capsule.	Stain by Gram.	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	Liquefaction—Form.	Line Growth.	Surface Growth.	Pellicle.	Turbidity.	Sediment.	Liquefaction.
...dent's Agar																										
...llus coli	1	4	+0.2										5	4	1	11	1	22	–							
		10											5	3	1	11	1	22	–							
	2	4	-0.1										2	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
	3	4	-0.2										6	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
	4	4	0.0										6	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
	5	4	+0.2										6	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
	6	4	+0.2										6	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
	7	4	-0.2										6	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
	8	4	+1.0										6	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
	9	4	0.0										6	1	1	11	1	22	–							
		10											6	1	1	11	1	22	–							
...llus cyaneus	1	4	+0.2										1	3	1	57	1	30	–							
		10											1	3	1	52	1	30	–							
	2	4	-0.1										2	4	1	11	1	30	–							
		10											2	3	1	51	1	30	–							
	3	4	-0.2										3	1	1	11	1	30	–							
		10											3	1	1	11	1	30	–							
	4	4	0.0										1	4	1	51	1	30	–							
		10											1	3	1	51	1	30	–							
	5	4	+0.2										1	4	1	51	1	30	–							
		10											1	3	1	51	1	30	–							
	6	4	+0.2										1	3	1	11	1	30	–							
		10											1	3	1	11	1	30	–							
	7	4	-0.2										1	3	1	51	1	30	–							
		10											1	3	1	57	1	30	–							
	8	4	+1.0										1	3	1	51	1	30	–							
		10											1	3	1	57	1	30	–							
	9	4	0.0										2	4	1	51	1	30	–							
		10											2	4	1	51	1	30	–							

Table 33

NAME.	INVESTIGATOR. Agar No.	Day of observation	Reaction	TYPE NUMBER.	MORPHOLOGY. Diameter + 1 micron.	Form in Rod. Spores.	Germination.	Capsule.	Stain by Gram	Grows at 20°.	Grows at 37°.	Fac. Anaerobe.	AGAR. Form of Growth.	Elevation of Growth.	Lustre.	Chromogenesis.	Topography of Growth.	Optical Characters.	Viscosity.	GEL. Liquefaction—Form.	Line Growth.	Surface Growth.	BROTH. Pellicle.	Turbidity.	Sediment.	S Liquefaction
dent's Agar																										
llus pyocyaneus	1	4	+0.2	–									3	1	1	11	1	22	–							
		10		95									1	1	1	11	1	22	–							
	2	4	-0.1	–									1	4	1	11	1	22	–							
		10		96									1	1	4	11	1	22	–							
	3	4	-0.2	95									1	1	4	11	1	30	–							
		10		95									1	1	4	60	1	30	–							
	4	4	0.0	95									1	1	4	60	1	30	–							
		10		95									1	1	4	60	1	30	–							
	5	4	+0.2	95									1	1	4	60	4	30	–							
		10		96									1	1	4	60	4	30	–							
	6	4	+0.2	–									1	4	1	11	1	22	–							
		10		97									9	1	1	11	1	22	–							
	8	4	+1.0	95									1	1	1	60	5	30	–							
		10		97									3	1	4	22	5	30	–							
	9	4	0.0	95									9	1	1	60	3	30	–							
		10		97									9	1	4	60	3	30	–							

REMARKS.

FOLD OUT

Table 34

FOLD OUT

Pathogenesis—Human.

REMARKS.

Table 35

FOLD OUT

Phenol.	Pathogenesis—Human.	REMARKS.

Table 36

FOLD OUT

Pathogenesis—Human.

REMARKS

Table 37

FOLD OUT

Phenol.	Pathogenesis—Human.	REMARKS

FOLD OUT

MILK.	FERMENTATION	NITRATE.		Phenol.	Pathogenesis—Human.	REMARKS.

Table 59

FOLD OUT

Pathogenesis—Human.

REMARKS.

Table 40

FOLD OUT

REMARKS

Table 41

FOLD OUT

Pathogenesis—Human.

REMARKS

Table 42

MILK. | FERMENTATION | NITRATE.

Pathogenesis | Vitality

REMARKS.

FOLD OUT

Table 13

FOLD OUT

REMARKS

FOLD OUT

REMARKS.

Table 45

FOLD OUT

REMARKS

Table 46

FOLD OUT

REMARKS

Table 47

25 - - I

REMARKS

FOLD OUT

Table 48

FOLD OUT

REMARKS.

Table 49

FOLD OUT

REMARKS.

Table 50

FOLD OUT

Phenol.	Pathogenesis—Human.	REMARKS.

Table 51

FOLD OUT

Pathogenesis—Human.

REMARKS.

Table 57

FOLD OUT

Pathogenesis—Human.

REMARKS.

Table 53

FOLD OUT

REMARKS.

Table 5d

ac

NA

REMARKS.

FOLD OUT

Table 55

cill

N

REMARKS.

FOLD OUT

Table 56

acill

REMARKS.

NAM

FOLD OUT

Table 57

illus

FOLD OUT

Pathogenesis—Human.

REMARKS.

Table 58

MILK.	FERMENTATION	NITRATE.			REMARKS.

FOLD OUT

Table 59

FOLD OUT

REMARKS.

Table 60

FOLD OUT

REMARKS.

Table 64